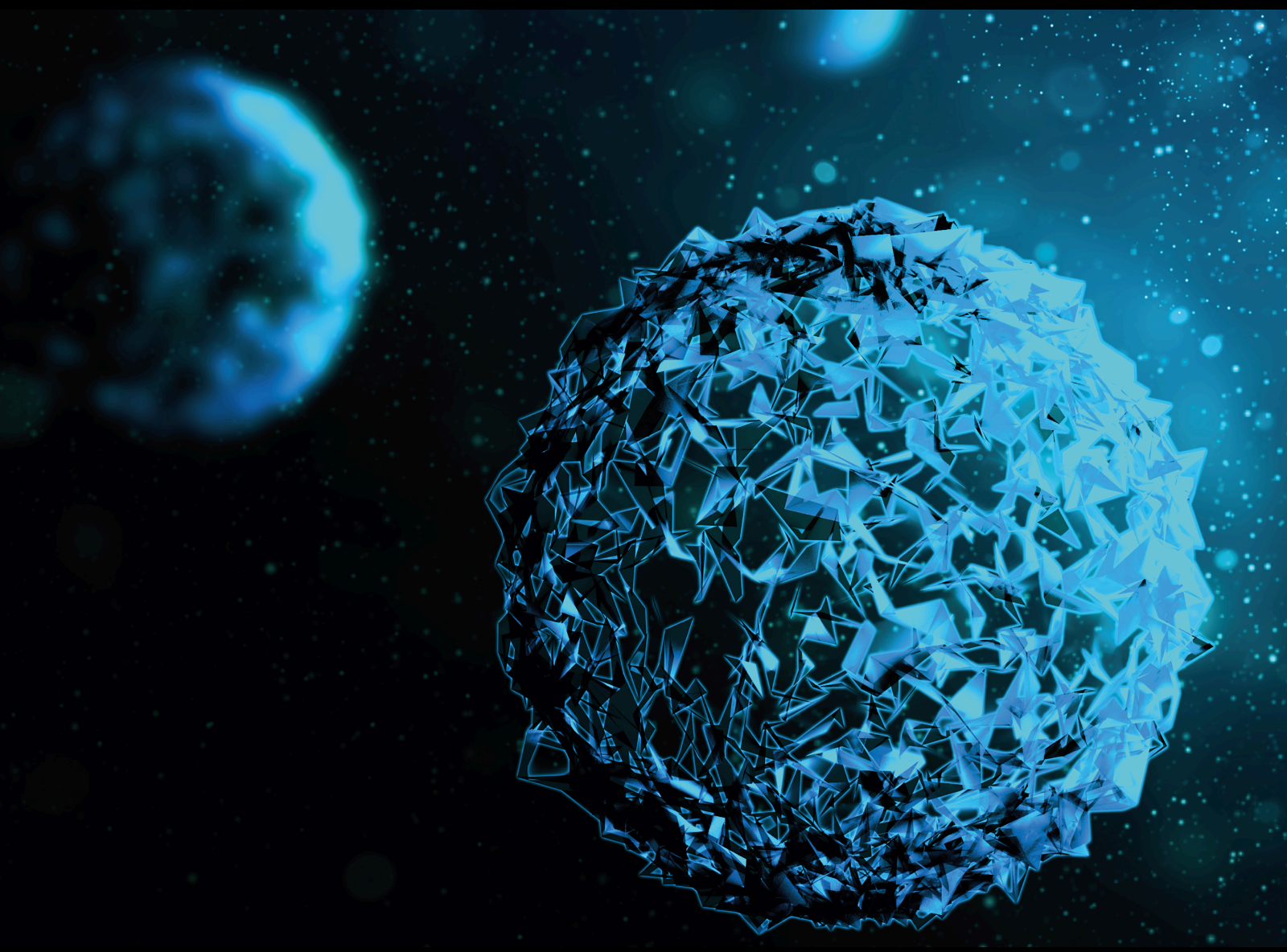
BioMed Research International

Recent Advances in Investigation, Prevention, and Management of Healthcare-Associated Infections (HAIs)

Lead Guest Editor: Yatao Liu
Guest Editors: Jin Long, Yiming Li, and Sharon F. Welbel



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Research Article

Analysis of Air Purification Methods in Operating Rooms of Chinese Hospitals

Bingli Zhang,[1] Liuyi Li,[1] Xi Yao,[1] Yuxiu Gong,[2] Yu Zhang,[2] Huai Yang,[3] Weiguang Li,[4] Ling Lin,[5] Yun Yang,[6] Haojun Zhang,[7] and Huixue Jia[1]

[1]*Peking University First Hospital, Beijing 100034, China*
[2]*National Institute of Hospital Administration, Beijing 100034, China*
[3]*Guizhou Provincial People's Hospital, Guiyang 550002, China*
[4]*Shandong Provincial Hospital, Jinan 250021, China*
[5]*Heilongjiang Provincial Center for Disease Control and Prevention, Harbin 150030, China*
[6]*Shanxi Dayi Hospital, Shanxi Academy of Medical Sciences, Taiyuan 030001, China*
[7]*Gansu Provincial Hospital, Lanzhou 730000, China*

Correspondence should be addressed to Liuyi Li; lucyliuyi@263.net

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Guest Editor: Yatao Liu

This research demonstrates the current use of air purification methods in the operating rooms (ORs) in China. 154 hospitals from 6 provinces were included in this survey to reflect the air purification methods of ORs in 2017. Air cleaning technology (ACT) is used in 124 (80.52%) hospitals. We find that the rates of using grade I, III, or IV clean operating room (COR) in tertiary hospitals are all higher than in lower level hospitals; the rate of using ACT in the ORs is higher, too. In addition, general hospitals have higher rate in using ACT in the ORs than specialized hospitals. The highest rate of using ACT in the ORs is in the eastern region of China. The number of hospitals using ACT, ultraviolet light disinfection, and air sterilizers (such as circulating air UV sterilizer) increased yearly. All grades of CORs can be maintained as required by more than 90% hospitals except grade II COR. In this research, we found air purification methods, especially the ACT, are widely used in hospitals' ORs. However, finding the way to select and use different air purification methods correctly is an urgent problem to be solved next.

1. Introduction

Health care-associated infection (HAI) affects the quality of medical care and the safety of health care workers and patients. It also brings a financial burden to the patients and hospitals [1–4]. Surgical-site infection (SSI) is one of the most common HAIs with high morbidity and mortality [5–7]. A study of SSI involving four kinds of surgeries (colorectal surgery, abdominal hysterectomy, femoral neck repair surgery, and vascular surgery) in 29 hospitals in China represented a SSI rate of 1.6% in 2014 [8]; this rate was higher than 0.9% in the US (reported by the National Healthcare Safety Network (NHSN), 2014) [3]. A recent study in the Netherlands also showed that the increased cost of SSI is reaching to €21,569 per case and total costs of DALYs for the three surgery types (colectomy, mastectomy, and total hip arthroplasty) exceeded to €88 million [9].

Researchers report that air microbial contamination in the OR is one of the risk factors for SSI [5, 10]. In 2016, the World Health Organization (WHO) released "Global Guidelines for the Prevention of Surgical Site Infection." They pointed out the importance of improving the air quality of the OR to prevent SSIs [11]. By properly and rationally using air purification methods, the air quality of ORs can be improved and contribute to reduce the risk of SSI to a certain extent [12, 13]. In 1972, a study on airborne contamination and the deep infection rate published by Charnley showed that, when the ventilation and microbiological performance of the OR were improved, the infection

rate would reduce from 7% to 0.5% [14]. Many studies suggested that laminar air flow (LAF) systems and ultraviolet air disinfection in ORs can significantly reduce the SSI rate [15, 16].

In order to regulate the air purification methods in hospitals in China, the Chinese government promulgated the standard "Management Specification of Air Cleaning Technique" in 2012. This standard is aimed for regulating the air purification methods in ORs and usage requirements [17]. The standard has been implemented for more than six years. During this time, many hospitals have undergone significant changes in air purification methods in ORs and new air purification methods have emerged. However, during this period, most of the studies are focusing on the effect of reducing SSIs, only a few of them focused on the status of air purification methods in ORs. Learning the types and composition of air purification methods used in ORs is necessary for the management and the development of air purification methods. In order to understand the current status of air purification methods in China and provide a basis for the management of air purification methods, we conducted this investigation nationwide.

2. Materials and Methods

2.1. Sampling Methods. A stratified sampling was conducted in six provinces from three regions of China (East, Central, and West). Provincial capital and two prefectural or municipal level cities were selected in each province. We selected 8~9 hospitals from each city for investigation, including at least one prefectural or municipal level general hospital, one district or county level general hospital, and one specialized hospital. Besides, 1 provincial or ministerial level general hospital was investigated in each province.

2.2. Data Collection and Management. The questionnaire was revised and improved by reviewing of the literature and consulting the experts, and then the unified design questionnaire was sent to the surveyed hospitals via a professional questionnaire network. We collected the basic information of hospitals including the hospital level, hospital type, number of beds, annual surgery volume, and air purification methods of ORs in 2017, distribution of various air purification methods in different years, and maintenance of air purification methods. After the reporting of data, the trained staff reviewed the data for errors and inconsistencies and verified data by telephone return visit. In addition, we verified the data reported by hospitals of two provinces in the field investigation. Unverifiable data were processed by "unknown."

2.3. Definitions

2.3.1. Air Cleaning Technology (ACT). Air cleaning technology is a technique that reduces airborne contaminants concentration by delivering air through a high-efficiency air filter and can be divided into turbulent flow and laminar flow according to the air distribution [13, 18].

2.3.2. Clean Operating Room (COR). It is the operating room where the total amount of microorganisms and dust particles in the operating environment air are reduced to an allowable level by using ACT. The classification of clean operating room is shown in Table 1 [19].

2.3.3. Chinese Hospital Level. The evaluation system of medical institutions implemented in China divided medical institutions into three levels (tertiary, secondary, and first) through comprehensive evaluation of service quality, technical level, and management level [20]. Some hospitals have not participated in the evaluation yet.

2.4. Data Analysis. Categorical variables are expressed as absolute numbers and their relative frequencies. The χ^2 test and Bonferroni method were used to test the differences on rates of using different air purification methods among hospital types, hospital levels, and regions. All statistical analyses were two-sided, and $p < 0.05$ was considered significant. Also, SPSS, version 22.0 was used for data analysis.

3. Results

A total of 154 hospitals were investigated, including 12 (7.79%) provincial or ministerial level hospitals, 82 (53.25%) prefectural or municipal level hospitals, and 60 (38.96%) county level hospitals. The characteristics of investigated hospitals, such as hospital level, hospitals type, region, no. of beds, and annual surgery volume, are shown in Table 2.

Among the 154 hospitals, ACT was the most common air purification method in the OR (124 hospitals (80.52%)). Among the 124 hospitals using ACT in the OR, 46 (37.10%) hospitals built only one grade of COR, and 78 (62.90%) built more than one grade of COR (Tables 3 and 4).

The rate of using ACT in the OR of tertiary hospitals was higher than that of lower level hospitals ($p < 0.05$), and we found the rates of using grade I COR, grade III COR, and grade IV COR were higher in tertiary hospitals ($p < 0.05$). The rate of using ACT in the OR in general hospitals was higher than the rate in specialized hospitals ($p < 0.05$), and the difference is mainly caused by the different rate of the grade I COR. Compared with the central and western regions in China, respectively, there were higher rates of using ACT in the OR in the eastern region ($p < 0.05$), and further analysis for different grade of CORs found that the rate of grade I COR was higher in the eastern region than that in other regions ($p < 0.05$) (Tables 5 and 6).

From 2001 to 2017, the number of hospitals using ACT, ultraviolet light disinfection, circulating air UV air sterilizer, electrostatic adsorption air sterilizer, and central air conditioning system with air purification device in ORs gradually increased year by year. Among these air purification methods, the number of hospitals using ACT in ORs always occupied top place every year from 2000 to 2017 and

Table 1: Classification of clean operating room.

Grade	Airborne bacterial concentration (at rest)	
	Operating zone (cfu/m^3)	Surrounding zone (cfu/m^3)
I	5	10
II	25	50
III	75	150
IV	175	

Table 2: Characteristics of surveyed hospitals.

Characteristics	No. of hospitals	Proportion (%)
Hospital level		
Tertiary	91	59.09
Secondary and lower	63	40.91
Hospital type		
General	117	75.97
Specialized	37	24.03
Region		
East	53	34.41
Central	50	32.47
West	51	33.12
No. of beds		
<400 beds	38	24.67
400~899 beds	51	33.12
>900 beds	65	42.21
Annual surgery volume		
<2000	39	25.32
2000~	47	30.52
6000~	68	44.16
Total	154	100.00

increased from 4 (2.60%) in 2000 to 118 (76.62%) in 2017 (Figure 1).

In more than 90% hospitals, grade I CORs, grade III CORs, and grade IV CORs can meet the requirement, while three of the four hospitals can maintain grade II CORs as required. All of the surveyed hospitals can maintain circulating wind UV sterilizer, ultraviolet light disinfection, electrostatic adsorption air sterilizer, and central air conditioning ventilation system with air purification device and mechanical ventilation as required. (Table 7).

4. Discussion

Since the first promulgation of the Architectural Technical Code for Hospital Clean Operating Department (GB50333) [19] in 2002, the number of hospitals with CORs has increased significantly in China. Provincial or municipal hospitals and even county level hospitals have begun to establish large-scale, high-standard CORs [21, 22]. This survey found, as of 2017, more than 80% of the hospitals have adopted ACT. Among them, more than 50% of hospitals have built the grade I COR and grade III COR, and most of hospitals with CORs are concentrated in economically developed eastern region. It can be seen that the ACT has become the most widely used air purification method in ORs in China nowadays. And, the number of hospitals using ACT in ORs has increased rapidly year by year, especially the grade I COR and the grade III COR. Standard drafter Jinming Shen once said that the misinterpretation for the norms is one of the reasons for accelerating the construction of CORs. He stressed that the purpose of formulating Architectural Technical Code for Hospital Clean Operating Department was to regulate the construction of the COR rather than to cancel the general OR [23]. In this survey, only four hospitals used the grade II COR, which were much less than the number of hospitals with other grades CORs. Owing to the standard Architectural Technical Code for Hospital Clean Operating Department (GB50333) [15], the requirements for the main technical indicators, basic equipment, layout, indoor decoration, air cleaning and conditioning system, etc. of the grade I COR and those of the grade II COR are almost identical; therefore, many hospitals preferred to build grade I CORs with higher cleanliness level.

ACTs have been widely used in ORs of China, but the effect of ACT on preventing SSIs, especially the LAF, has been controversial [11, 24–26]. In 2016, the WHO released the "Global Guidelines for The Prevention of Surgical Site Infection" [11], the guidelines recommend that "the panel suggests that laminar airflow ventilation systems should not be used to reduce the risk of SSI for patients undergoing total arthroplasty surgery," but it also mentioned that because the data used in the study were not specifically designed for LAF effects on SSIs, it may be affected by factors such as the number of hospitals, surgeons, characteristics, or implementation of patients admitted. And, the guidelines also mentioned that the single studies found that LAF has different effects on the risk of SSI for different types of surgeries.

In addition, there are also differences in the recommended cleanliness level of the OR for different types of surgeries among countries. In the United States, the American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE) standard 170-2017 "Ventilation of Health Care Facilities" [27] mentioned that high-risk special operating room is recommended for large-scale surgery requiring general or large-area local anesthesia and vital function maintenance equipment, which is equivalent to grade I COR in China. The standard Architectural Technical Code for Hospital Clean Operating Department (GB50333) [15] in China recommended grade I COR for prosthesis, implantation, some large organ transplants, and surgery with SSI which will directly endanger life and quality of life.

At last, the construction costs on CORs and the maintenance costs of LAF are extremely high. A study in Italy concluded that construction costs of CORs have increased by 24% and annual operating costs have increased by 36% [28]. Domestic research indicated the annual operating cost of a grade I COR can reach more than 100,000 yuan [21, 29, 30]. The construction and maintenance of CORs can increase the national medical expenditure, and the unqualified construction and inadequate maintenance of CORs will make the air cleaning equipment a source of airborne microbial contamination [31, 32], and the low-price bidding policy in China further affected the construction quality of

Table 3: Air purification methods in operating rooms of surveyed hospitals.

Air purification methods	No. of hospitals	Proportion (%)
ACT	124	80.52
Circulating wind UV sterilizer	7	4.54
Electrostatic adsorption air sterilizer	6	3.90
Central air conditioning system with air purification device	6	3.90
Ultraviolet light disinfection	6	3.90
Ultraviolet light disinfection and air sterilizer*	3	1.94
Mechanical ventilation	2	1.30
Total	154	100.00

*Air sterilizer included electrostatic adsorption air sterilizer, circulating wind UV sterilizer, and plasma air sterilizer.

Table 4: Different grade clean operating rooms of surveyed hospitals.

Different grades of COR	No. of hospitals	Proportion (%)
I and III	50	40.32
III	24	19.35
I	19	15.32
I, III, and IV	17	13.71
III and IV	7	5.65
IV	3	2.42
I, II, and III	2	1.61
II and III	1	0.81
I, II, III, and IV	1	0.81
Total	124	100.00

Table 5: Analysis of air clean technology in operating rooms of surveyed hospitals.

Characteristics	Total no. of hospitals	No. of hospitals using ACT in ORs (%)	χ^2	p value
Hospital level			**13.04**	**<0.01**
Tertiary	91	82 (90.11)		
Secondary and lower	63	42 (66.67)		
Hospital type			**7.61**	**0.01**
General	117	100 (85.47)		
Specialized	37	24 (64.86)		
Region			**7.34**	**0.03**
East	53	49 (92.45)		
Central	50	37 (74.00)		
West	51	38 (74.51)		

Table 6: Analysis of different grade clean operating room in surveyed hospitals.

Characteristics	No. of hospitals	No. of hospitals using the COR I (%)	χ^2	p value	No. of hospitals using the COR III (%)	χ^2	p value	No. of hospitals using the COR IV (%)	χ^2	p value
Hospital level			**9.75**	**<0.01**		**11.37**	**<0.01**		**4.76**	**0.03**
Tertiary	91	62 (68.13)			70 (76.92)			23 (25.27)		
Secondary and lower	63	27 (42.86)			32 (50.79)			7 (11.11)		
Hospital type			**23.36**	**<0.01**		0.36	0.55		1.11	0.29
General	117	80 (68.38)			79 (67.52)			25 (21.37)		
Specialized	37	9 (24.32)			23 (62.16)			5 (13.51)		
Region			**6.42**	**<0.05**		0.47	0.79		1.36	0.51
East	53	38 (71.70)			37 (69.81)			13 (24.53)		
Central	50	25 (50.00)			32 (64.00)			8 (16.00)		
West	51	26 (50.98)			33 (64.71)			9 (17.65)		

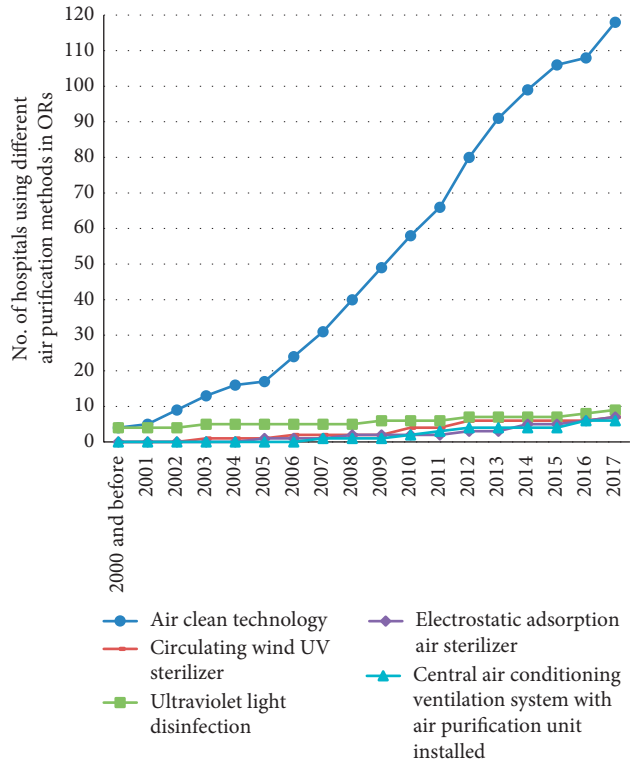

Figure 1: Distribution of various air purification methods in different years. The data are missing for 6 hospitals using ACT in ORs and missing for 1 hospital using circulating wind UV sterilizer in ORs.

Table 7: Analysis of the maintenance of different air purification methods in surveyed hospitals' ORs.

Air purification methods	No. of hospitals	No. of hospitals with clean maintenance (%)	No. of hospitals replacing key components as required (%)
Grade I of ACT	89	86 (96.63)	85 (95.51)
Grade II of ACT	4	3 (75.00)	3 (75.00)
Grade III of ACT	102	97 (95.10)	99 (97.06)
Grade IV of ACT	30	29 (96.67)	29 (96.67)
Ultraviolet light disinfection	9	9 (100.00)	9 (100.00)
Circulating wind UV sterilizer	8	8 (100.00)	8 (100.00)
Electrostatic adsorption air sterilizer	7	7 (100.00)	7 (100.00)
Central air conditioning ventilation system with air purification device	6	6 (100.00)	6 (100.00)
Mechanical ventilation	2	2 (100.00)	2 (100.00)

CORs and maintenance [33]. Faced with these questions, should the hospital continue to build CORs and how to properly use ACT in ORs? Hu [33] believed that hospitals should establish CORs according to their own scale, mission requirements, nature, etc. A study has shown the general OR with the proper air purification method can also effectively control the number of bacterial colonies and dust in the air [28]. There have been rules for the construction of general ORs in China. The standard GB15982-2012 Hygienic Standard for Disinfection in Hospitals [34] defined clearly the environmental sanitation requirements of the general OR; the standard GB51039-2014 Code for Design of General Hospital [35] provided relevant regulations for the architectural design and heating, ventilation and air conditioning systems of the general OR; the standard WS/T368-2012 Management Specification of Air Cleaning Technique in Hospitals [17] had listed the air purification methods available for the general OR.

With the development of science and technology, new air purification methods are constantly appearing. The survey found that, in addition to ACT, the number of hospitals using circulating air UV sterilizers and electrostatic adsorption air sterilizers in ORs has been slowly increased year by year. Research has shown that using the dynamic air sterilizer in the OR can reduce the impact of personnel activity on air quality to a certain extent [36]. However, various temperature and humidity in different hospitals caused by the vast territory of China and the different density of personnel in different hospitals lead to that the disinfection effect of one air purification method in different

hospitals is diverse. The standard WS/T648-2019 General Hygienic Requirements for Disinfecting Machine [37] issued by Chinese government on March 1, 2019, made requirements for the disinfection effect of air sterilizers and further standardized the sanitary requirements for air sterilizers. Nevertheless, the air disinfection effect of one air purification method in various situations remains to be further studied. At the same time, we found ultraviolet light disinfection is still an important air disinfection method in ORs. Ultraviolet light disinfection has been proven to reduce the level of contamination in ORs and thereby preventing the SSI, but there are issues such as the frequency, amount, and locations for ultraviolet light disinfection needed to be further researched [16]. The survey also found that the use rate of circulating air UV sterilizers has gradually increased to 5.19% in 2017, which was close to that of ultraviolet light disinfection (5.84%). Studies have shown that there was no significant difference in the air disinfection effect between two purification methods under static conditions, but the total amount of microorganisms in the OR is rapidly increased under dynamic conditions after ultraviolet light disinfection [38, 39]. As mentioned above, air disinfection effects of different air purification methods are affected by a lot of factors, so further research is needed.

There are still many doubts about the selection of air purification methods. For example, there is no clear standard for situations requiring the construction of a COR, no rules for surgery type which can be operated in a general OR, and so on. All of the above issues require further research to provide scientific evidence-based evidence for the formulation and revision of national policies. Last but not the least, using air purification methods is just one of the ways to improve the air quality of the ORs. Strengthening the comprehensive management of the OR also plays an important role in improving and maintaining the quality of the air in the OR, including the development of the corresponding management system, controlling the number and the state of the personnel in ORs, the good management between surgeries, controlling the times and time of opening the surgical door, and cleaning and disinfection of the environment. [40–43]. Therefore, we should take comprehensive measures to improve the cleanliness of the OR and reduce the risk of SSI.

5. Conclusion

The number of hospitals using ACT in ORs has been increasing year by year. The rate of using different grades of CORs varies according to hospital level, region, and hospital type. Other air purification methods, including ultraviolet light disinfection, circulating wind UV sterilizer, and electrostatic adsorption air sterilizer also widely used in hospitals' ORs. How to correctly select and use different air purification methods is an urgent problem to be solved.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

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Review Article

Probiotics Used for Postoperative Infections in Patients Undergoing Colorectal Cancer Surgery

Chongxiang Chen,[1,2] Tianmeng Wen ,[3] and Qingyu Zhao [1]

[1]*Department of Intensive Care Unit, Sun Yat-sen University Cancer Center, State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Guangzhou 510060, China*
[2]*Guangzhou Institute of Respiratory Diseases, State Key Laboratory of Respiratory Disease, the First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510120, China*
[3]*School of Public Health, Sun Yat-sen University, Guangzhou, Guangdong Province, China*

Correspondence should be addressed to Qingyu Zhao; zhaoqy163@163.com

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Objective. The objective of this study was to conduct a systematic review and meta-analysis about probiotics to improve postoperative infections in patients undergoing colorectal cancer surgery. *Methods*. The PubMed and the Web of Science were used to search for appropriate randomized clinical trials (RCTs) comparing probiotics with placebo for the patients undergoing colorectal cancer surgery. The RevMan 5.3 was performed for meta-analysis to evaluate the postoperative infection, including the total infection, surgical site infection, central line infection, pneumonia, urinary tract infection, septicemia, and postoperative leakage. *Results*. Our meta-analysis included 6 studies involving a total of 803 patients. For the incidence of total postoperative infection (odd ratios (OR) 0.31, 95% confidence interval (CI) 0.15–0.64, $I^2 = 0\%$), surgical site infection (OR 0.62, 95% CI 0.39–0.99, $I^2 = 11\%$), central line infection (OR 0.61, 95% CI 0.15–2.45, $I^2 = 65\%$), pneumonia (OR 0.36, 95% CI 0.18–0.71, $I^2 = 0\%$), urinary tract infection (OR 0.26, 95% CI 0.11–0.60, $I^2 = 26\%$), septicemia (OR 0.28, 95% CI 0.17–0.49, $I^2 = 10\%$), postoperative leakage (OR 0.45, 95% CI 0.06–3.27, $I^2 = 68\%$), the results showed that the incidences of infections were significantly lower in the probiotics group than the placebo group. *Conclusions*. Probiotics is beneficial to prevent postoperative infections (including total postoperative infection, surgical site infection, pneumonia, urinary tract infection, and septicemia) in patients with colorectal cancer.

1. Introduction

The postoperative complications of colorectal cancer surgery result in increased ventilation days, hospital stay days, mortality, and cost. Postoperative infection is a major factor affecting the morbidity of the patients. Bacterial translocation is defined as transmitting the bacteria from the gastrointestinal tract to normally sterile tissues. A large number of studies have shown that the bacterial translocation plays a significant role in increasing the incidence of postoperative infections [1, 2].

The probiotics therapy, which was introduced by Lilly and Stillwell [3], could lead to positive clinical and laboratorial outcomes for patients undergoing gastrointestinal surgery. Probiotics are live microorganisms and it is known that probiotics benefit to the host as they can stabilize the intestinal microbiological environment. Nowadays, probiotics have been proved to treat several diseases, such as chronic inflammatory bowel disease [4], hepatic encephalopathy [5], and atopic disease [6]. Horvat et al. [7] showed us his interesting finding that preoperative administration of prebiotics in elective colorectal surgery had the same protective effect in preventing a postoperative inflammatory response as mechanical bowel cleaning.

Probiotics study is very important in recent year, there is a recent paper discussing about the importance of probiotics in the prevention and treatment of colorectal cancer. So we want to conduct a meta-analysis to integrate all this interesting studies to guide clinical practice, as meta-analysis has the higher quality than common RCTs if we only include high quality RCTs. We try to explore the incidence of post-operative infections, including the incidence of the total infection and subgroup infection, such as surgical site infection, central line infection, pneumonia, urinary tract infection, septicemia and postoperative leakage.

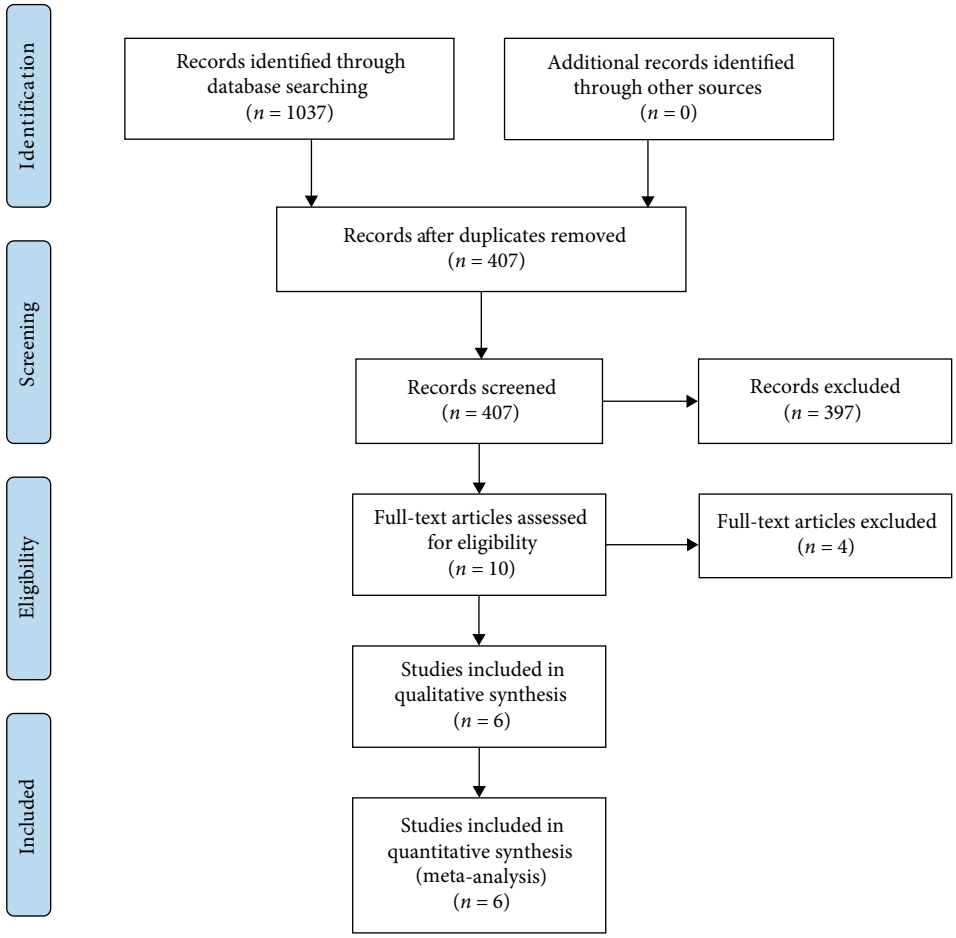

Figure 1: Flow diagram of choosing the appropriated articles.

2. Methods

2.1. Search Strategy. Two investigators independently searched the articles in the databases (PubMed, the Web of Science). The reference lists of eligible studies and relevant papers were also manually searched and reviewed. Searching terms included "probiotics", "colorectal cancer", and "surgery". Searching terminal date was 2019/1/10. Firstly, we found 407 articles after duplications excluded, and then 307 of them were excluded by reading the title and abstract. Finally, 6 articles were left after reading the whole articles [8–13] (Figure 1).

2.2. Inclusion and Exclusion. Inclusions contain: (1) randomized controlled study comparing probiotics with placebo, (2) outcome: various kinds of infections, (3) only be published in English.

Exclusions contain: (1) review, retrospective research, case report, (2) insufficient data in the articles.

2.3. Data Elected. Two authors (Chongxiang Chen, Tianmeng Wen) independently reviewed the identified abstracts and selected articles to full review. The third reviewer addressed the discrepancies (Qingyu Zhao). For each selected publication, the following baseline and study characteristics were extracted: first author, publication year, country, participant characteristics, patient age, dosage form of probiotics groups, experimental durations, and the baseline characteristics of these studies were concluded (Table 1). The risk of bias of the included studies is shown in Figures 2 and 3. Efficacy outcome measures were the total infection, surgical site infection, central line infection, pneumonia, urinary tract infection, septicemia, and postoperative leakage.

2.4. Risk of Bias Assessment. The risk of bias of trials included in this meta-analysis was assessed according to the recommendations of the Cochrane Handbook of Systematic Reviews of Interventions, in the following domains: selection bias (random sequence generation and allocation concealment), performance bias (blinding of participants and personnel), detection bias (blinding of outcome assessment), attrition bias (incomplete outcome data), and reporting bias (selective outcome reporting) (http://handbook.cochrane.org). Jadad scale was used to calculate the quality of every enrolled study.

2.5. Statistic Analysis. We pooled data and used mean deviation (OR, with 95% confidence interval) for dichotomy outcomes: the total infection, surgical site infection, central line infection, pneumonia, urinary tract infection, septicemia, and postoperative leakage. We would use a fixed-effect model

Table 1: Baseline characteristics of these studies.

Study	Type	Jadad scale (randomization + concealment of allocation+double blinding + withdrawals and dropouts)	Time (published)	Country	Participant	Total number (probiotics vs. placebo)	Age (probiotics vs. placebo)	Probiotics	Duration
Kotzampassi et al.	RCT	1 + 1 + 2 + 1 = 5	2015	Greece	One center	164; 84/80	Total: ≥18 years; 65.9 ± 11.5 vs. 66.4 ± 11.9	One capsule (Lactobacillus acidophilus LA 5, Lactobacillus plantarum, Bifidobacterium lactis BB 12 Saccharomyces boulardii) twice a day	The day of operation and for the next 14 days
Liu et al.	RCT	2 + 1 + 2 + 1 = 6	2012	China (Taiwan)	One RCC	150; 75/75	Total: 25–75 years; 66.06 ± 11.02 vs. 62.28 ± 12.41	Encapsulated bacteria (Lactobacillus plantarum; Lactobacillus acidophilus11; Bifidobacterium longum 88) patients in probiotics group received 2 g/d, at a total daily dose of 2.6×10^{14} CFU	6 days preoperatively and 10 days postoperatively
Liu et al.	RCT	2 + 2 + 2 + 1 = 7	2015	China	One ICU	134; 66/68	Total: 25–75 years; 66.62 ± 18.18 vs. 60.16 ± 16.20	Encapsulated probiotics (Lactobacillus plantarum; Lactobacillus acidophilus11; Bifidobacterium longum 88); patients in probiotics group received 2 g/d, at a total daily dose of 2.6×10^{14} CFU	Intervention period lasted 16 days, 6 days preoperatively and 10 days postoperatively; Detailed records were recorded for up to 30 days after surgery
Sadahiro et al.	RCT	1 + 1 + 0 + 1 = 3	2013	Japan	One ICU	195; 100/95	Total: 20-80 years; 67 ± 9 vs. 66 ± 12	Three Bifidobacteria tablets orally after each meal three times daily	For 7 days before the operation and from postoperative day 5 for 10 days
Zhang et al.	RCT	1 + 1 + 2 + 1 = 5	2011	China	One center	60; 30/30	Total: None 61.5 (46–82) vs. 67.5 (45–87)	3 oral bifid triple viable capsules (Enterococcus faecalis; Lactobacillus acidophilus; Bifidobacterium longum), 3 times a day	For 3 days (days −5 to −3) before surgery
Liu et al.	RCT	2 + 2 + 2 + 1 = 7	2010	China	One center	100; 50/50	Total: 25–75 years; 65.3 ± 11 vs. 65.7 ± 9.9	Encapsulated bacteria (contain: Lactobacillus plantarum; Lactobacillus acidophilus; Bifidobacterium longum); patients in placebo group received probiotics 2 g/d, total daily dose of 2.6×10^{14} CFU	6 days preoperatively and 10 days postoperatively

RCT: randomized controlled trial; ICU: intensive care unit; RCC: respiratory care center.

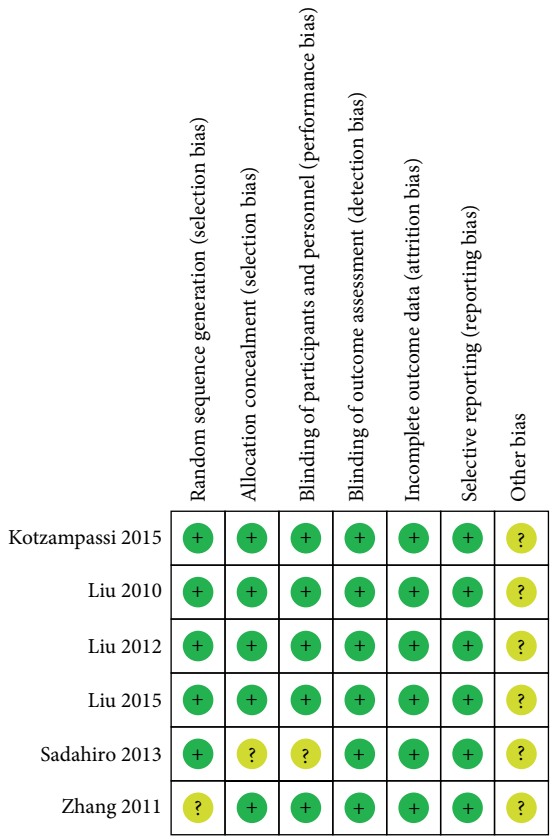

Figure 2: Risk of bias summary.

if there were no considerable heterogeneity among the studies. We would use a random-effects model if the I^2 statistic was above 50% and Cochran's Q statistic had a P value ≤0.1. Funnel plots were used to screen for potential publication bias. All statistical analyses were carried out with Review Manager 5.3 (The Cochrane Collaboration).

3. Results

The studies included in our meta-analysis were all randomized controlled trials, published from 2010 to 2015. The studies were conducted in Greece [11], China [8, 9, 12, 13], and Japan [10]. Table 1 presents the basic characteristics of included trials and demographic data of participants. All trials were one-center studies and the Jadad Scales of all included studies ranged from 3 to 7.

3.1. Total Infection. Comparing probiotics with placebo, our study showed that probiotics could significantly decrease the incidence of postoperative infections. For the total postoperative infection, our study included 3 studies with a total of 220 patients; the results by comparing groups were significantly lower in probiotics group (odd ratios (OR) 0.31, 95% confidence interval (CI) 0.15–0.64). Heterogeneity testing showed that I^2 = 0%, indicating low heterogeneity (Figure 4).

3.2. Surgical Site Infection. For the incidence of surgical site infection, our study included 6 studies involving a total of 653 patients, and the result demonstrated that probiotics was significantly lower than placebo (OR 0.62, 95%CI 0.39–0.99, I^2 = 11%). Heterogeneity testing showed that I^2 = 11%, indicating low heterogeneity (Figure 5).

3.3. Central Line Infection. For the results of the incidence of central line infection, our study enrolled 2 studies, including a total of 284 patients, central line infection (OR 0.61, 95%CI 0.15–2.45, I^2 = 65%) reflected no significant difference in two groups. Heterogeneity testing showed that I^2 = 65%, indicating high heterogeneity (Figure 6).

3.4. Pneumonia. For the incidence of pneumonia, our study enrolled 4 studies, including a total of 508 patients, and the result showed that probiotics was significantly lower than the placebo (OR 0.36, 95%CI 0.18–0.71, I^2 = 0%). Heterogeneity testing showed that I^2 = 0%, indicating low heterogeneity (Figure 7).

3.5. Urinary Tract Infection. For the result of the incidence of urinary tract infection, our study included 3 studies and a total of 448 patients, and the result reflected significant difference in groups (OR 0.26, 95%CI 0.11–0.60, I^2 = 26%). Heterogeneity testing showed that I^2 = 26%, indicating low heterogeneity (Figure 8).

3.6. Septicemia. For the result of the incidence of septicemia, our study enrolled 4 studies, including a total of 509 patients, and the result showed that probiotics was significantly lower than the placebo (OR 0.28, 95%CI 0.17–0.47, I^2 = 10%). Heterogeneity testing showed that I^2 = 10%, indicating low heterogeneity (Figure 9).

3.7. Postoperative Leakage. For the result of the incidence of postoperative leakage, our study enrolled 3 studies, including a total of 419 patients, and the result did not show that probiotics was significantly lower than the placebo (OR 0.45, 95%CI 0.06–3.27, I^2 = 68%). Heterogeneity testing showed that I^2 = 68%, indicating high heterogeneity (Figure 10).

Potential publication bias of probiotics used for surgical site infection was performed and shown as funnel plot (Figure 11).

4. Discussion

Several RCTs showed that the use of probiotics in patients with abdominal surgery was a promising approach to the prevention of postoperative infectious complications and well tolerated by patients with minor side effects [14]. However, in abdominal surgery, some investigators reported that there was no evidence supporting any benefit of a preoperative use of probiotics in patients with critical illnesses and undergoing elective abdominal surgery with increased risk of mortality [15, 16], and that in some cases, there was even an increased risk of mortality.

In our meta-analyses, the results showed that probiotics could effectively decrease the rate of postoperative infections, such as pneumonia, surgical site infection, and urinary tract infection.

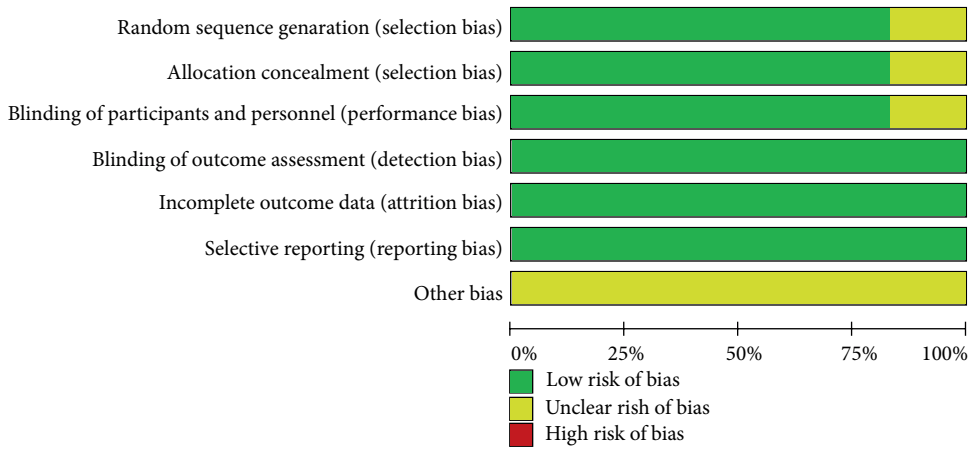

Figure 3: Risk of bias graph.

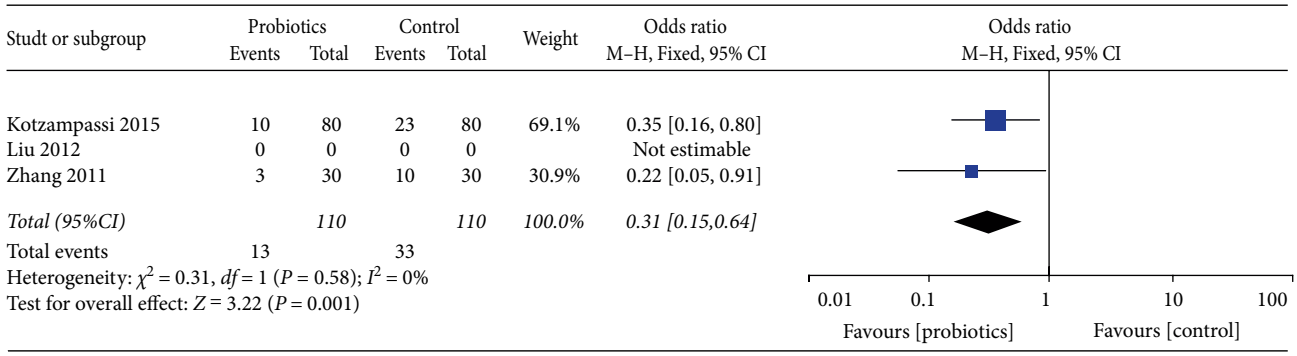

Figure 4: Incidence of total infection.

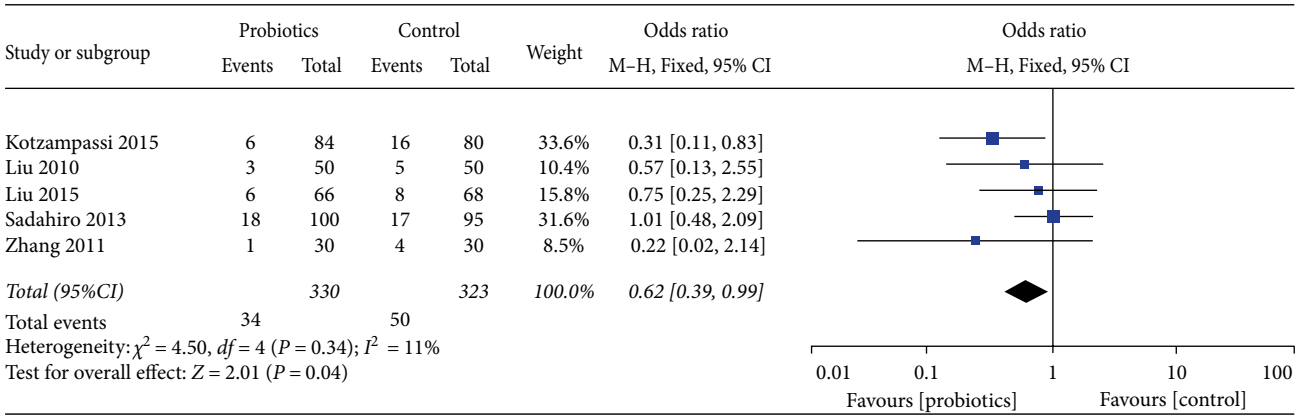

Figure 5: Incidence of postoperative surgical site infection.

Not only the incidence of infections but also the quality of life is improved in these studies, which shortens the duration of postoperative hospital stay and the antibiotics administration period. Furthermore, probiotics are considered to generate antitumor agents, which have chemo-preventive effects against colorectal cancer [17]. In addition, probiotics can improve immune function [18].

It is shown that probiotics protect epithelial barrier function. The outcomes probably result from the balance of the enteral bacteria environment. The use of probiotics after surgery markedly improved intestinal microbial populations and significantly decreased the incidence of further infectious complications. The mechanism of the action of probiotics may be related with either the earlier bowel movement preventing bacterial translocation from the gut or the modulation of the innate immune responses.

Gastrointestinal microbiota may be modulated by probiotics. Our study demonstrated that the use of probiotics improved the capacity of the gut ecosystem to survive from surgically induced injury, resulting in fewer postoperative

Study or subgroup	Probiotics Events	Probiotics Total	Control Events	Control Total	Weight	Odds ratio M–H, Fixed, 95% CI
Liu 2012	4	75	12	75	49.5%	0.30 [0.09, 0.96]
Liu 2015	7	66	6	68	50.5%	1.23 [0.39, 3.86]
Total (95%CI)		*141*		*143*	*100.0%*	*0.61 [0.15, 2.45]*
Total events	11		18			

Heterogeneity: $\tau^2 = 0.66$; $\chi^2 = 2.87$, $df = 1$ ($P = 0.09$); $I^2 = 65\%$
Test for overall effect: $Z = 0.70$ ($P = 0.48$)

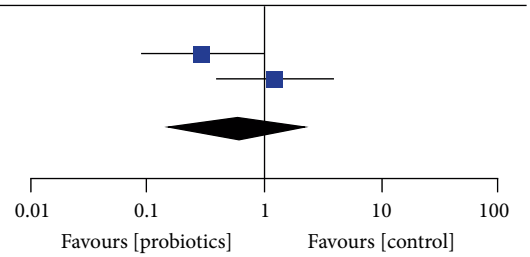

Figure 6: Incidence of postoperative Central line infection.

Study or subgroup	Probiotics Events	Probiotics Total	Control Events	Control Total	Weight	Odds ratio M–H, Fixed, 95% CI
Kotzampassi 2015	2	84	9	80	30.4%	0.19 [0.04, 0.92]
Liu 2012	3	75	10	75	32.4%	0.27 [0.07, 1.03]
Liu 2015	6	66	8	68	24.2%	0.75 [0.25, 2.29]
Zhang 2011	1	30	4	30	13.0%	0.22 [0.02, 2.14]
Total (95%CI)		*255*		*253*	*100.0%*	*0.36 [0.18,0.71]*
Total events	12		31			

Heterogeneity: $\chi^2 = 2.62$, $df = 3$ ($P = 0.45$); $I^2 = 0\%$
Test for overall effect: $Z = 2.92$ ($P = 0.003$)

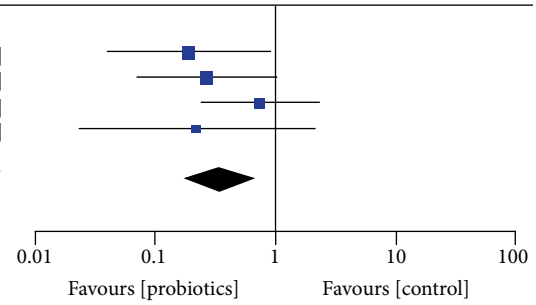

Figure 7: Incidence of postoperative pneumonia.

Study or subgroup	Probiotics Events	Probiotics Total	Control Events	Control Total	Weight	Odds ratio M–H, Fixed, 95% CI
Kotzampassi 2015	4	84	6	80	24.1%	0.62 [0.17, 2.27]
Liu 2012	2	75	10	75	40.0%	0.18 [0.04, 0.84]
Liu 2015	1	66	9	68	35.9%	0.10 [0.01, 0.82]
Total (95%CI)		*225*		*223*	*100.0%*	*0.26 [0.11, 0.60]*
Total events	7		25			

Heterogeneity: $\chi^2 = 2.71$, $df = 2$ ($P = 0.26$); $I^2 = 26\%$
Test for overall effect: $Z = 3.11$ (P = 0.002)

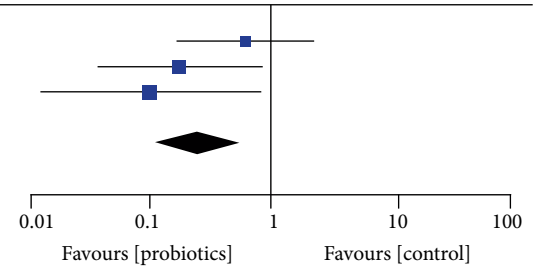

Figure 8: Incidence of postoperative urinary tract infection.

Study or subgroup	Probiotics Events	Probiotics Total	Control Events	Control Total	Weight	Odds ratio M–H, Fixed, 95% CI
Kotzampassi 2018	1	85	4	80	6.7%	0.23 [0.02, 2.07]
Liu 2012	41	75	55	75	40.9%	0.44 [0.22, 0.87]
Liu 2015	39	66	60	68	39.7%	0.19 [0.08, 0.47]
Zhang 2011	1	30	8	30	12.7%	0.09 [0.01, 0.82]
Total (95%CI)		*256*		*253*	*100.0%*	*0.28 [0.17,0.47]*
Total events	82		127			

Heterogeneity: $\chi^2 = 3.33$, $df = 3$ ($P = 0.34$); $I^2 = 10\%$
Test for overall effect: $Z = 4.94$ ($P < 0.00001$)

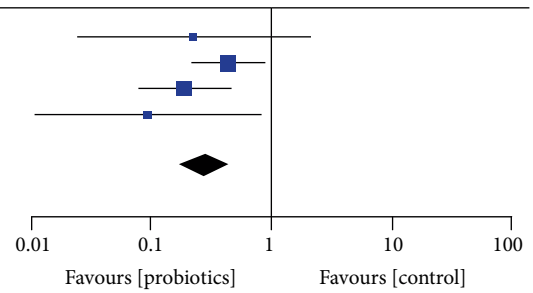

Figure 9: Incidence of postoperative septicemia.

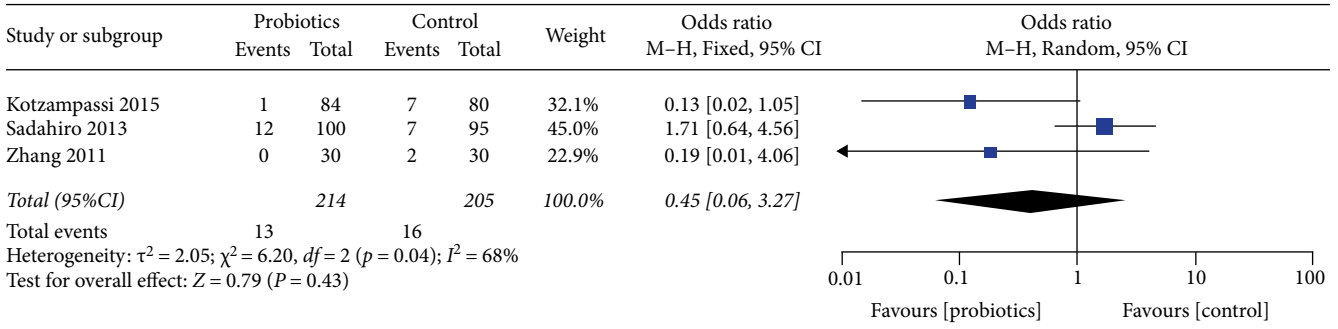

Figure 10: Incidence of postoperative leakage.

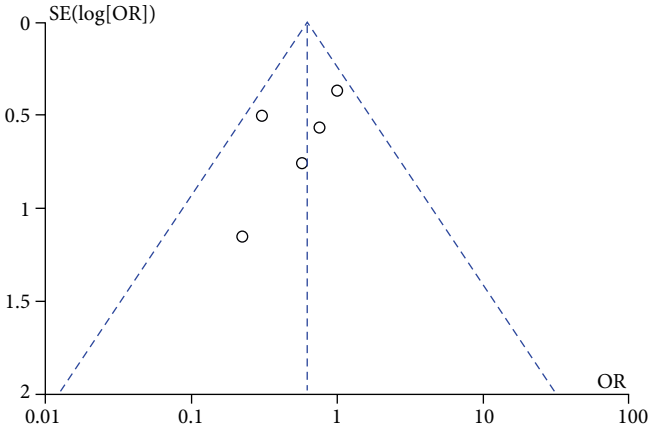

Figure 11: Funnel plot of surgical site infection.

infections. Thus, we concluded that maintaining gut microbiota balance and diversity is important for enhancing host defenses, especially during the recovery from major surgery.

The drawbacks of our study are described as follows: Firstly, we only searched the studies written by English, and the total subjects included in our study were less than the study conducted by Ouyang et al. [19]. Moreover, the combination with prebiotics was not taken into account. Furthermore, the probiotics strain, dose and dosage form in collected studies were not consistent, and the probiotics treatment was combined with other pretreatment modes in some studies, which probably induced certain confusing factors.

However, our study calculated the results of the exactly total subgroups of infection, including the pneumonia, septicemia, central line infection, surgical site infection, postoperative leakage, and urinary tract infection. So our study contains more comprehensive viewpoints of the benefit of probiotics used in colorectal cancer patients undergoing surgery.

5. Conclusion

All in all, probiotics is beneficial to prevent postoperative infections (including total postoperative infection, surgical site infection, pneumonia, urinary tract infection, and septicemia) in patients with colorectal cancer. We recommend perioperative oral intake of probiotics as the treatment in patients needing gastrointestinal surgery.

Data Availability

The datasets used and/or analyzed in the current study are available from the corresponding author upon request.

Consent

All authors have agreed to the publication of this manuscript.

Conflicts of Interest

The authors declare that they have no coflicts of interest.

Authors' Contributions

Chongxiang Chen designed the study. Chongxiang Chen, Qingyu Zhao designed the search strategy and performed the search. Chongxiang Chen, and Tianmeng Wen performed abstract screening, full text screening, data extraction, and risk of bias assessment. Chongxiang Chen and Qingyu Zhao drafted the manuscript. All authors revised the manuscript, as well as reading and approving the final manuscript.

Acknowledgments

We acknowledge contribution of all authors.

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Research Article

Characteristics and Management of Spinal Tuberculosis in Tuberculosis Endemic Area of Guizhou Province: A Retrospective Study of 597 Patients in a Teaching Hospital

Peng Wang,[1] **Wenbo Liao**,[1,2] **Guangru Cao**,[2] **Yongyan Jiang**,[3] **Jingcheng Rao**,[3] **and Yi Yang**[3]

[1]*Department of Orthopaedics, The Affiliated Hospital of Zunyi Medical University, Huichuan District, Dalian Road, Zunyi, Guizhou, China*
[2]*Department of Orthopaedics, The Second Affiliated Hospital of Zunyi Medical University, Xinpu New Area, Zunyi, Guizhou, China*
[3]*Zunyi Medical University, Huichuan District, Dalian Road, Zunyi, Guizhou, China*

Correspondence should be addressed to Wenbo Liao; wenbo900@sina.com

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Background. Tuberculosis (TB) is an endemic disease in Guizhou. Spinal TB accounts for approximately 50% cases of skeletal TB. The purpose of this study was to investigate the characteristics and management of patients treated for spinal TB in a certain hospital and to provide guidance for the prevention and treatment of spinal TB. *Methods*. The clinic records of all patients diagnosed with spinal tuberculosis in a teaching hospital between January 2010 and December 2018 were collected. The epidemiology, clinical characteristics, imaging and laboratory findings, treatment methods, and prognosis were recorded and analyzed. *Results*. During this nine-year period, 597 patients with spinal TB were identified. There were 313 males and 284 females with an average age of 43 years. The largest number of patients fell in the age group of 21–30 years; mean time from symptom onset to diagnosis in the hospital was 17 months. Back pain was the main clinical manifestation (89.34%). The most common imaging technique was computed tomography (CT, 96.80%), followed by magnetic resonance imaging (MRI, 84.01%). Majority of the lesions involved the lumbar spine (47.30%), followed by the thoracic spine (40.95%). 178 (29.82%) patients in this study had varying degrees of neurological impairment. 22.78% of the patients selected conservative treatment, and surgical treatment was performed in 483 patients (80.90%). *Conclusions*. The incidence of spinal TB was generally on the rise throughout the study period. After diagnosed with spinal TB, all patients got appropriate treatment and achieved good efficacy, but most of the patients did not pay much attention to the disease and receive timely treatment. Thus, it is essential to strengthen the TB preventive strategies, improve the health awareness of residents and universal resident health examination.

1. Introduction

As a result of acquired immunodeficiency syndrome (AIDS) and new drug-resistant strains of tuberculosis (TB), the resurgence of spinal TB (STB) has sparked a flurry of activity toward the prevention and treatment of this condition [1]. Despite advances in the methods of diagnosis and treatment, it is still a global public health problem. According to the World Health Organization (WHO), globally, the best estimate is that 10.0 million people (range, 9.0–11.1 million) developed TB in 2017, and the disease burden caused by TB is falling globally, but not fast enough to reach the first (2020) milestones of the End TB Strategy [2]. China's fifth TB epidemiological survey shows that the prevalence of TB in the western region is higher than the central and eastern regions. In Guizhou Province, located in the western region, the economic development is lagging behind, so TB prevention and control started late. Zunyi City has a high incidence and is the key area of prevention and control of TB in Guizhou Province; the total incidence of TB ranked first in

the legal infectious diseases because the strategy of the Directly Observed Therapy Shortcourse (DOTS strategy) covering the province was late, leading to poor control and high epidemic of TB. As a common disease of spine, there are a number of publications regarding STB locally and internationally [3,4], but few studies on STB have been conducted in Guizhou. In order to better implement the DOTS strategy, we need to further understand the epidemiological characteristics, diagnosis, and current status of treatment of STB in the region at this moment. This study intends to retrospectively analyze the clinical features, diagnosis, and treatment of 597 cases of patients with STB in a tertiary teaching hospital from January 2010 to December 2018 at Zunyi City, Guizhou Province, and to provide a reference for the prevention and treatment of STB.

2. Materials and Methods

We retrospectively reviewed the medical records of patients admitted for STB to the Orthopaedics Department in the Affiliated Hospital of Zunyi Medical University between January 2010 and December 2018 by medical record coding searches. The epidemiology, clinical characteristics, imaging and laboratory findings, treatment methods, and prognosis were recorded. All patients have no autoimmune diseases and non-HIV infected. Diagnosis was established following clinical manifestations and radiological and haematological examination, supplemented by postoperative pathological examination of biopsy specimens. The composite reference standard (CRS) used for categorization of patients into 4 groups: confirmed TB cases (culture positive, smear negative/culture positive, or smear positive/culture positive), probable TB cases (culture negative but showing clinical symptoms, radiological fifindings, and/or histology/cytology suggestive of TB), possible TB cases (negative culture and other tests and only clinical symptoms and/or signs suggestive of TB; in this group, the patient follow-up indicated response to empirical ATT after 3 months), and not TB (culture and all other tests for TB were negative, and patient improved without receiving TB treatment) (Table 1) [5].

3. Results

3.1. Demographics. The number of patients receiving treatment for STB per year increased after 2010. During this nine-year period, according to the CRS, 597 patients with probable TB were admitted to the Affiliated Hospital of the Zunyi Medical University and categorized into the clinical diagnosis group, which included 313 males (52.43%) and 284 females (47.57%) and their mean age was 43 years (range, 13–89 years) and male to female ratio was 1.10. The largest number of patients fell in the 21–30 years age group (125, 20.94%), followed by 41–50 and 31–40 years age group, accounting for 17.59% and 16.75% of total STB patients, respectively. Mean time from symptom onset to diagnosis in the hospital was 17 months (range, 1 day–240 months) (Table 2).

Risk factors consisted of smoking in 247 patients (41.37%), hypertension in 28 patients (4.69%), hepatitis B in 14 patients (2.35%), and diabetes in 9 patients (1.50%), of which one patient developed diabetic nephropathy. Thirty-one patients had a previous history of TB, including STB (15 cases), pulmonary TB (8 cases), elbow TB (5 cases), and renal TB (3 case). At the time of diagnosis, 183 patients (30.65%) had concomitant pulmonary TB. Thirty-eight cases had other extrapulmonary site involvement, including TB of kidney (15 cases), tuberculous meningitis (8 cases), TB of joint (6 cases), cervical lymph node TB (2 case), epididymis TB (2 cases), tuberculocele (1 case), TB of rib (1 case), fallopian tubes TB (1 case), bladder TB (1 case), and spleen TB (1 case). None of the patients were HIV-positive and immunosuppressing conditions or treatments (e.g., steroid therapy, chemotherapy, etc.).

3.2. Clinical Manifestations. Most of the patients were admitted to the hospital because of constitutional symptoms; six patients were diagnosed with STB due to health examination, five patients obtaining medical advice due to pathological fracture caused by spinal TB. Among all the symptoms, back pain was the most common clinical complaint (534, 89.45%), followed by sweating (184, 30.82%), motor weakness (165, 27.64%), numbness (149, 24.96%), weight loss (138, 23.12%), and low-grade fever (133, 22.28%). At physical examination, tenderness was found in 465 patients (77.89%), percussion pain in 420 patients (70.35%) and kyphosis in 183 patients (30.65%). Neurological status was evaluated according to the Frankel classification (Table 3). A total of 178 (29.82%) of the 597 patients had a neurological deficit: Frankel A in 18 patients, Frankel B in 23 patients, Frankel C in 52 patients, and Frankel D in 85 patients (Table 4), of which one patient in Frankel C had a tumor in the infection site, and the pathology after surgery was confirmed as schwannoma.

3.3. Imaging Findings. All patients were admitted to the hospital after X-ray examination at the outpatient department. In addition, the most common imaging technique used to evaluate the patients for spinal lesions was CT (96.80%), followed by MRI (84.01%). Radionuclide bone scanning was performed only in 15 patients, all of who had foci of increased uptake. The imaging study consistently suggested that paraspinal abscesses were visible in 439 patients (73.53%). The 597 patients had 1620 lesions, the most commonly involved site was the thoracic vertebral (813, 50.19%), followed by the lumbar vertebral (698, 40.09%), while the sacral vertebral (67, 4.14%) and cervical vertebral (42, 2.59%) were less commonly involved (Figure 1). Approximately, a quarter of patients (145, 24.29%) had three or more vertebral bodies involved, and multiple level skip lesions were seen in 25 (4.19%) cases (Table 5), of which the thoracolumbar spine was the most common site. There were eighteen patients with pathological fracture caused by STB.

3.4. Laboratory Test Results. Hemoglobin (normal range is 115–150 g/L) had a routine investigation and in our study ranged from 61 to 164 g/L; the percentage of patients with

Table 1: Algorithm for patient categorization into different categories of the composite reference standard.

CRS category	Result					
	AFB smear	Culture	Symptoms/signs[a]	Radiology[b]	Histology/cytologyun[c]	Follow-up at 3 mo[d]
Confirmed TB	+/−	+	+	+/−	+/−	+
Probable TB	+/−	−	+	+	+	+
	+/−	−	+	+	−	+
	+/−	−	+	−	+	+
Possible TB	+/−	−	+	−	−	+
Not TB	−	−	+	−	−	−

[a]Weight loss, persistent cough, and fever for 2 to 3 weeks. [b]For radiology, a specimen was positive if the presence of infiltrates or cavities, hilar lymph nodes, pleural effusions, or tuberculomas was noted. [c]For histology/cytology, a specimen was positive if the presence of caseation necrosis with epitheloid granulomas was reported irrespective of the visual presence or absence of acid-fast bacilli. [d]For follow-up at 3 months, a specimen was positive if the patient was on antitubercular treatment (ATT) and negative if the patient responded to non-ATT.

Table 2: Characteristics of 597 spinal TB patients.

Characteristics	Value
Age (years)	43 (13–89)
Age distribution (years) *N* (%)	
13~20	55 (9.21)
21~30	125 (20.94)
31~40	100 (16.75)
41~50	105 (17.59)
51~60	86 (14.07)
61~70	99 (16.58)
71~80	23 (3.85)
81~89	4 (0.67)
Sex, *N* (%)	
Male	313 (52.43)
Female	284 (47.57)
Smoking	247 (41.37)
Comorbidities, *N* (%)	
Pulmonary TB	183 (30.65)
Hypertension	28 (4.69)
Renal TB	15 (2.51)
Hepatitis B	14 (2.35)
Diabetes mellitus	9 (1.50)
Meninges TB	8 (1.34)
Duration of symptoms	17 (1d~240 m)

Table 3: Neurological grades of frankel scale definition.

Frankel A: complete motor and sensory loss below the lesion
Frankel B: incomplete, some sensory loss below the lesion
Frankel C: incomplete, motor and sensory sparing, but the patient is not functional
Frankel D: incomplete, motor and sensory sparing, but patient can stand and walk
Frankel E: normal, complete functional recovery

Table 4: Clinical symptoms and signs of 542 spinal TB patients.

Characteristics	Value (%)
Clinical symptoms	
Back pain	534 (89.45)
Night sweats	184 (30.82)
Weakness	165 (27.64)
Numbness	149 (24.96)
Weight loss	138 (23.12)
Fever	133 (22.28)
Clinical signs	
Tenderness	465 (77.89)
Percussion pain	420 (70.35)
Kyphosis	183 (30.65)
Frankel classification, *N* (%)	
A	18 (3.01)
B	23 (3.85)
C	52 (8.71)
D	85 (14.24)

hemoglobin between 90 and 115 g/L was 44.56%, less than 90 g/L accounting for 5.12%. In addition, the ESR was measured in most patients (578, 96.82%) and ranged from 1 to 140 mm/h.

113 patients had less than 20 mm/h, and 161 patients had greater than 100 mm/h. 563 patients with CRP content ranged from 0.17 to 207.51 mg/l; the CRP was normal in 21.11% of patients, but others increased in varying degrees.

3.5. Treatments. Among the 597 patients, 136 patients (22.78%) were chosen for conservative treatment (quadruple antituberculosis agents: isoniazid 0.3 g/d, rifampicin 0.45 g/d, ethambutol 0.75 g/d, and pyrazinamide 0.75 g/d), of which 22 patients were rehospitalized for surgical treatment after antituberculous chemotherapy 2 weeks later. After enough antituberculosis therapy and admission in the hospital, surgical treatment was performed in 483 patients (80.90%) and achieved good efficacy [6] (Figure 2). Anterior debridement was performed in 22 patients (3.69%) with an average length of hospital stay for 25.85 ± 2.3 days. Lateral-anterior debridement, bone grafting, and internal fixation were performed in 261 patients (43.72%) with an average length of hospital stay for 24.11 ± 1.8 days. Posterior debridement, bone grafting, and internal fixation were performed in 90 patients (15.08%) with an average length of hospital stay for 21.37 ± 2.5 days. Anterior debridement, bone grafting, and posterior internal fixation was performed in 86 patients (14.41%) with an average length of hospital stay for 24.24 ± 2.3 days. Among all the patients, there were 24 cases (4.02%) who underwent minimally invasive surgery, anterior or posterior decompression, bone grafting, and posterior internal fixation using the endoscopic system with an average length of hospital stay for 22.00 ± 1.3 days. Of all

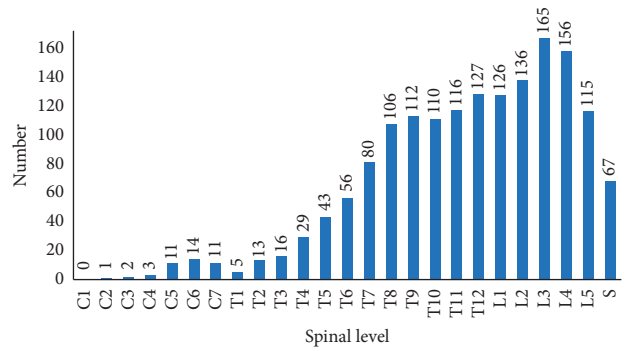


Figure 1: Number of patients with vertebrae involved at each spinal level.

Table 5: Imaging characteristics and laboratory findings of 597 spinal TB patients.

Characteristics	Value, *n* (%)
Location	
Cervical	20 (2.70)
Thoracic	303 (40.95)
Lumbar	350 (47.30)
Sacral	67 (9.05)
Vertebra involved	
1	92 (15.41)
2	360 (60.30)
≥3	145 (24.29)
Skipped lesion	
C, T	2 (0.34)
T, L	13 (2.18)
T, T	4 (0.67)
L, S	3 (0.50)
T, L, S	3 (0.50)

(C-cervical vertebral; T-thoracic vertebral; L-lumbar vertebral; S-sacral vertebral).

the surgical patients, two patients were undergoing twice additonal hospitalization and surgery because of the twice internal fixation fracture after the first surgery. Eleven patients were enrolled again after surgery due to sinus formation, of which 3 patients underwent focal debridement and drainage, and 8 patients were healed by strengthening wound dressing replacement.

4. Discussion

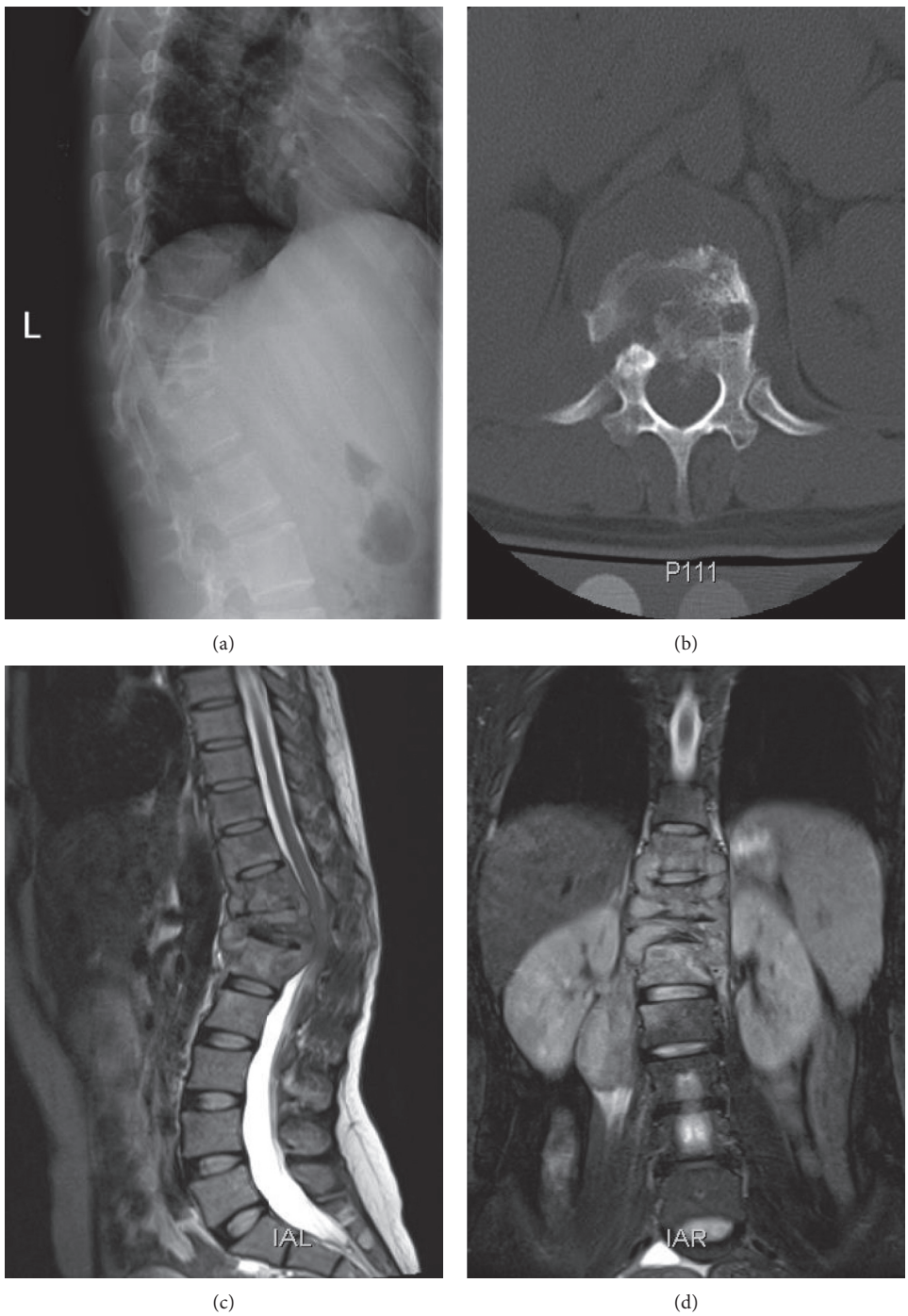
STB was first described in 1782 by Percival Pott [7]. It accounts for 50% cases of skeletal TB [8]. And 1%–3% of patients suffering from TB have involvement of the skeletal system [9]. Thoracic and lumbar spine is the most common site of involvement, incident in the population of about 3%–5% [10], and is one of the primary causes of spinal deformity and paralysis. As the most common form of skeletal TB, with the increasing incidence of TB, the incidence of STB increased year by year [11]. In our institution, from 2010 to 2018, the number of hospitalized patients with STB showed an overall upward trend. As Shi et al. reported [12], the largest number of patients in our subjects included the 21–30-year-old age group (20.94%). Considering the age of the patients just entered the society, greater mobility, harsh living conditions, and poor health awareness, leading to increased risk of TB infection. At the time of diagnosis, 21.11% of our patients are older than 60 years, which is the reason for the older average age compared to other studies and even have a diagnostic delay for about twenty years [3]. This phenomenon may be closely linked to the characteristics of Guizhou, the socioeconomic status, and poor health consciousness of patients.

STB is characteristically chronic and slowly progressive, more prevalent in immunocompromised persons. Unlike pulmonary TB, STB is seldom accompanied with symptoms such as cough, sputum, fever, body weight loss, or night sweating, which is not easy to attract the attention from patients and doctor and more easily misdiagnosed than pulmonary TB. Similar to other studies [13], back pain was the most common presenting symptom, followed by night sweats, body weight loss, and low-grade fever. Unlike in other studies [3], back pain, weakness, and numbness were the major symptoms. It is noteworthy that 6 patients were diagnosed with STB due to health examination, underwent surgical treatment after enough antituberculous chemotherapy, and recovered well after surgery. Although the number of such patients is less, the importance of health examination in the prevention and treatment of TB, especially in a high incidence area of TB, worthy of further promotion is shown.

Similar to Weng et al.'s study [9], in the current study, concomitant TB of the lung was present in 30.65% of patients. Therefore, people who have pulmonary TB should be strongly alert of the occurrence of spinal TB. In our study, 178 patients (29.82%) had neurological damage such as numbness, weakness, and varying degrees of hypoesthesia. McLain and Isada [14] reported that neurological deficits are common in thoracic and cervical involvement and, if untreated, may progress to complete and incomplete paraplegia, which may be closely related to the structure of the cervical and thoracic spine.

Early diagnosis allows rapid therapeutic intervention and prevention of possible complications. Disk space narrowing and vertebral body destruction are the most common changes seen on plain radiographs, which may however be normal at the earliest stage of the disease. Similar to other studies [4,15], the thoracic and lumbar spines were the spinal segments most commonly affected; in this study, 47.30% involved the lumbar spine and 40.95% involved the thoracic spine. In contrast to the report by Peto et al. [16], 50% of lesions were involved in the thoracic and lumbar spine. MRI and CT are still the most useful modalities for detecting spinal lesions, especially MRI, which has good sensitivity and specificity [17], shows lesions typical of discitis, detects epidural spread, and paraspinal abscesses earlier than other imaging studies [18].

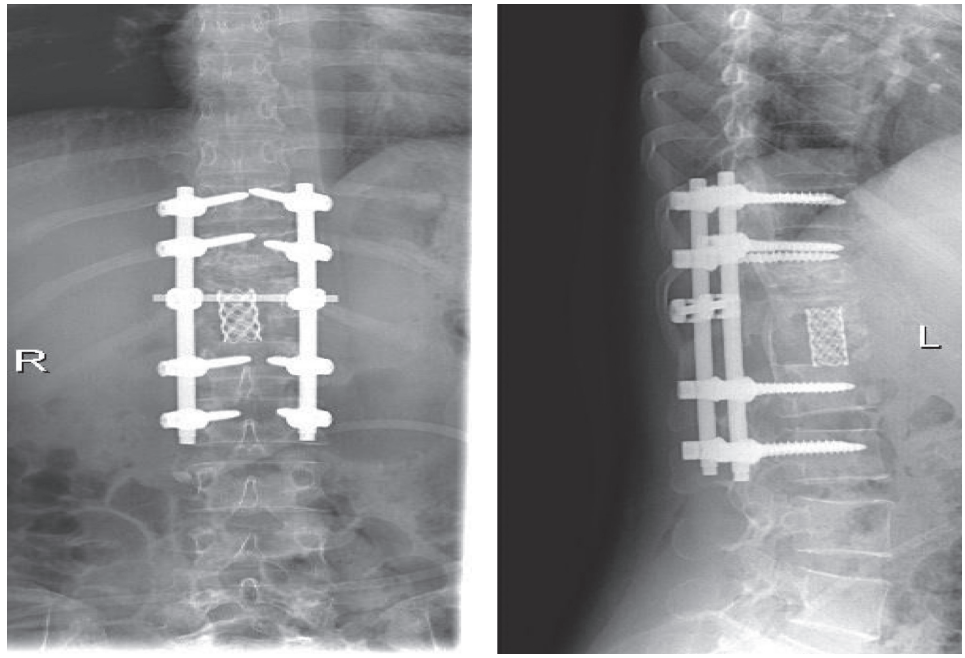
ESR and CRP are the most commonly used parameters to monitor the disease activity and follow up the therapeutic response of STB [19]. ESR is a very sensitive but a highly nonspecific test. In our study, ESR ranged from 1 to 140 mm/h, which was different from other authors' observation [20]. Similarly, the CRP level ranged from 0.17 to 207.51 mg/l;



(a) (b)

(c) (d)

Figure 2: Continued.



(e) (f)

Figure 2: (a) Radiograph of a 32-year-old woman showing T9-L2 vertebral destruction with kyphosis. (b) Axial CT scan showing T12 vertebral destruction. (c) Sagittal MRI showing T9-L2 vertebral destruction with kyphosis, paravertebral, and intraspinal abscesses. (d) Coronal MRI showing T9-L2 vertebral destruction with paravertebral and psoas abscesses. (e, f) A postoperative radiograph shows deformity corrected and satisfactory positioning of the internal fixation device.

Mulleman et al. [21] pointed out that the CRP level of 23 patients with STBranged from 6 to 197 mg/l. Sudprasert et al. [22] found that the mean value of CRP was 80.4 mg/L in the patients who had neurological deficit due to spinal TB and the earlier declination of CRP in postoperative was closely related to the neurological recovery.

Hemoglobin and albumin are indicators of human nutritional status; malnutrition is one of the important reasons for the development of STB [23]. Of all patients, 297 patients (49.75%) had anemia and 64 patients (10.72%) had hypoproteinemia. Therefore, nutrition support is important in the course of all treatments because STB is a wasting disease [24]. The World Health Organization also recommended the strengthening of nutritional support at the same time of anti-TB treatment [25]. In addition, the occurrence of STB is also closely related to the immune system, and some reports [26] have indicated that immunotherapies that could modulate the immune system and better control M. tuberculosis replication, shorten the course, and significantly improve therapeutic effect in patients with latent TB infection or active disease is a promising approach for host-directed therapy.

The management of STB consists of supportive care, chemotherapy, and surgery. Antituberculous chemotherapy remains the mainstay of therapy throughout the treatment process. The fundamental principle of chemotherapy in TB is that any regime chosen must include multiple drugs and must be given for a prolonged period of time. Therapy should be continued for a duration of 6 to 9 months [27]. Because of its longer cycle and serious adverse reaction, failure of chemotherapy and emergence of drug resistance are frequently due to the failure of compliance [28]. Chemotherapy alone, nevertheless, cannot correct problems arising from bone destruction. Therefore, despite the availability of effective conservative treatments, surgical procedures still assume an important role in the management of STB.

STB patients manifested neurological deficits due to compression of the spinal cord, cauda equina, or nerve root by tuberculous granulation tissue, abscess. Godlwana et al. [29] reported that 56% of their subjects presented with neurological deficits, in which 24% had complete paraplegia and 32% incomplete paraplegia, and the prognosis was closely related to the degree of deficits. 29.82% of patients in this study had varying degrees of neurological impairment. Anti-TB chemotherapy is necessary for surgical intervention for spinal TB. Adequate antitubercular chemotherapy combined with surgical treatment is important to save the spinal cord function and avoid irreversible neurological dysfunction. Surgical treatment can not only decompresses nerves, but also can control the infection, correct deformity, and reshape the stability of the spinal segment [30]. The efficacy of surgical treatment on STB has been confirmed. Anterior, posterior and combined techniques as well as osteotomies, vertebral column resection, have been described to correct spinal alignment and restore sagittal balance [31,32]. The indications for surgery were compression of the spinal cord, cauda equina, or nerve root; severe spinal kyphosis and progressive spinal kyphosis; spine instability; and compression of vital organs by huge abscess.

In our population, surgical treatment was performed in 483 patients (80.90%) after anti-TB chemotherapy proved to be effective. All patients' choice of procedure depends on the site and level of vertebral involvement, but generally it is radical lateral-anterior debridement, bone grafting, and internal fixation. At the same time, a part of the patients with STB chose minimally invasive surgical treatment and recovered well; with the development of minimally invasive conceptions, minimally invasive technology has become one of the standard treatment procedures for STB.

5. Conclusions

The incidence of STB was generally on the rise throughout the study period. After diagnosed, all patients received appropriate treatment and achieved good efficacy, but most of the patients did not pay much attention to the disease and receive timely treatment. Early diagnosis and prompt treatment of STB are necessary to prevent permanent neurological disability and to minimize spinal deformity. TB remains a major public health problem, and STB should also be widely concerned, especially in Guizhou where the socioeconomic and medical levels are lagging behind; people do not pay much attention to the disease. Thus, it is essential to strengthen the TB preventive strategies and improve the health awareness of residents.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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Research Article

Comparative Study of Microbiological Monitoring Results from Three Types of Sampling Methods after Gastrointestinal Endoscope Reprocessing

Su Ma,[1] **Lili Feng**,[2] **Ziyi Jiang**,[3] **Xian Gao**,[3] **Xisha Long**,[3] **Shaonan Zhuang**,[4] **Wenxia Ding**,[1] **Taiyao Chen**,[5] **Zhaoshen Li**,[1] **Lingjuan Zhang**,[4] **Huijun Xi**,[4] **and Hongzhi Zhang**[5]

[1]*Gastrointestinal Endoscopy Center, Changhai Hospital, Shanghai, China*
[2]*Department of Anesthesiology, Changhai Hospital, Shanghai, China*
[3]*School of Nursing, The Navy Military Medical University, Shanghai, China*
[4]*Department of Nursing, Changhai Hospital, Shanghai, China*
[5]*Shanghai Municipal Center for Disease Control and Prevention, Shanghai, China*

Correspondence should be addressed to Huijun Xi; xhj_cn@126.com and Hongzhi Zhang; wxws46@163.com

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Objective. Compare the effects of three sampling methods on the microbiological monitoring results after reprocessing of gastrointestinal endoscopes, providing scientific basis for improving the monitoring quality of gastrointestinal endoscope cleaning and disinfection. *Method.* Gastrointestinal endoscopes after reprocessing were selected randomly at the gastrointestinal endoscopy center of a tertiary hospital in Shanghai from October 2018 to February 2019. The endoscopes selected were all sampled in three different methods under continuous sampling and intermittent sampling respectively. Methods used includes, the biopsy channel group (Group A), the entire channel group (Group B), and the disc brush group (Group C). Then the colony forming units (CFU/piece) were counted in the laboratory. *Results.* A total of 12 endoscopes were sampled by using continuous sampling approach, in which the detection rate of bacteria in disc brush group (33.3%) and entire channel group (33.3%) was higher than biopsy channel group (8.3%). Among the 12 endoscopes sampled with intermittent approach, the detection rate of bacteria from high to low was the disc brush group (50%), the entire channel group (41.7%), and the biopsy channel group (8.3%). *Conclusion.* Different sampling methods will lead to the difference of microbiological culture results after reprocessing of gastrointestinal endoscope, indicating that the improved sampling method is beneficial to objectively reflect the endoscope cleaning and disinfection effect, and improve the monitoring quality of endoscope disinfection.

1. Introduction

In recent years, flexible endoscope reprocessing failure has been listed in the "Top 10 Health Technology Hazards" issued annually by the Emergency Care Research Institute (ECRI) for five consecutive years and even ranked the top one of the list in the year 2016. With the continuous advancement of gastrointestinal endoscopy technology, new endoscopic technique may bring new medical risks and medical technique hazards while improving medical quality and patient safety. According to the report, from 1996 to 2015, among 1389 cases of patients under duodenoscopy procedures in Europe, 32 cases were found of being infected with multidrug resistant *Escherichia coli* due to the failure of endoscopic reprocessing [1]; from October 3, 2014, to January 28, 2015, patients from UCLA Medical Center died of Carbapenem-resistant Enterobacter (CRE) infections obtained from contaminated endoscopy; in 2015, 186 patients were infected with Middle East Respiratory Syndrome due to endoscopic reprocessing failure, of which 19.4% died [2]. As per the data from the American Journal of Infection Control, 2018, endoscopes after reprocessing, including gastroendoscope, intestinal endoscope, duodenal endoscope, ultrasound endoscope, etc., the bacterial positive rate ranges from 60% to 92% [3], and in China from 2007 to 2012, the qualification rate after reprocessing of gastrointestinal endoscopes was only 80.8% [4]. Accordingly, the quality of the gastrointestinal endoscopic reprocessing is not stable, and scientific and objective

Figure 1: Flush the instrument channel from the control section (Group A).

monitoring is urgently needed. Meanwhile, more and more domestic and international guidelines emphasize the importance of endoscope microbial monitoring.

The microbiological monitoring can evaluate the effect and quality of endoscopic reprocessing, and is beneficial to identify sources of contamination, correct cleaning, and disinfection methods, and thus preventing the spread of nosocomial infections It is mentioned in the guidelines from Europe, Australia, and New Zealand [5]. For microbiological sampling of gastrointestinal endoscope, the APIC (Association for Professionals in Infection Control Epidemiology, 2000) recommends sampling the suction and biopsy channel as well as the air-water channel in a flushing method; the ESGE-ESGENA (European Society of Gastrointestinal Endoscopy and European Society of Gastroenterology and Endoscopy Nurses and Associates, 2008), Canada (2010) sets the rule that the endoscopes should be sampled with entire channel by antegrade flushing method. The GESA-GENCA (Gastroenterological Society of Australia and Gastroenterological Nurses College of Australia, 2010) recommends to sample the entire channel of endoscopes with the sequence of flush-brush-flush method in both antegrade and retrograde manner, and the UMCG (University Medical Center Groningen, 2011) suggests the sampling by retrograde rinsing of endoscopic suction biopsy and air-water channel [6]. In order to improve the detection rate of endoscopic microbial contamination after reprocessing, and to more objectively evaluate the effect of endoscopic reprocessing, the Ministry of Health of China has issued the national standard for endoscope cleaning and disinfection since 2004, established the requirement of sample frequency, channel should be sampled and fluid volume of samples. In 2016, newly issued "Regulation for cleaning and disinfection technique of flexible endoscope WS 507-2016" [7] updates and supplements bunches of quality control methods and details of endoscope cleaning and disinfection. The regulation emphasized the entire channel sample method, using 50 ml instead of 20 ml of eluate containing neutralizer, it also emphasized the total collection method, as well as bacteria culture by filter membrane method to improve the elution effect and detection efficiency. However, there are wide difference on sampling sites, sampling methods, and frequency, as well as evaluation indicators between different international guidelines [8]. It becomes an urgent problem and needs to be tackled on how to conduct a microbiological monitoring examination more scientifically, reasonably, and regularly. This study starts with the sampling method in gastrointestinal endoscopic microbiological monitoring, discusses the influence of different sampling methods on the culture results, and provides basis for further establishing scientific and accurate culture methods. The specific procedures are summarized as follows.

2. Materials and Methods

2.1. Experimental Material. A total of 12 gastrointestinal endoscopes were randomly selected from October 2018 to February 2019 from the gastrointestinal endoscopy center of a tertiary hospital in Shanghai, included 10 gastroscopes (Olympus, Japan), 2 colonoscopes (Olympus, Japan), and 24 disc brushes (Normandie Endo Technologies, France, general type). The neutralizer is an aldehyde neutralization enrichment medium (Haibo Biotechnology, China).

2.2. Experimental Methods

2.2.1. Endoscope Cleaning and Disinfection Method. According to the operation requirements of "Regulation for cleaning and disinfection technique of flexible endoscope WS 507-2016", every endoscope should be strictly reprocessed in accordance with the procedures of "precleaning, leak testing, washing, rinsing, disinfection, terminal rinsing, and drying".

2.2.2. Sampling Method. In order to avoid the deviation of the detection results due to the difference in the amount of bacteria contaminated between different endoscopes, two approaches were used in this experiment. One was to perform the biopsy channel sampling, the entire channel sampling, and disc brush sampling continuously on the same gastrointestinal endoscope in the same day for further testing, which was called as continuous sampling; the other was to perform biopsy channel sampling, entire channel sampling, and disc brush sampling on the same endoscope for three days respectively for further testing, which was called as intermittent sampling. The entire operating procedure followed the principle of aseptic technique. A peristaltic pump was used and an injection needle was repeatedly injected for 2-3 times for full amount collection.

(1) Biopsy channel sampling group (Group A): the endoscopes after reprocessed were sampled as below: 50 ml of the neutralizer was extracted with a sterile syringe, injected, and flushed the instrument channel through the biopsy port of the control section, and the total volume of elution was collected from the distal end of the endoscope. The eluate was thoroughly mixed and sent to the laboratory of Shanghai Municipal Center for Disease Control and Prevention within 2 hours for culturing and colony counting (CFU/piece) (Figure 1).
(2) Entire channel sampling group (Group B): the endoscopes after reprocessed were sampled as below: 50 ml of the neutralizer was extracted with a sterile syringe. A sterile film was placed onto the air/water port, suction, and instrument port of the endoscopic control

(a) (b) (c)

Figure 2: Flush the instrument and suction channel from the suction port (Group B). (a) Close the instrument port, (b) close the air/water and suction port, and (c) flush the suction port.

(a) (b) (c)

Figure 3: Brush-flush the instrument channel from the instrument port (Group C). (a) Brush the instrument channel, (b) cut off the brush, and (c) cut into the bottle.

section. Installed the sterile endoscope specialized washing joint to seal the air/water injection port, suction port; and instrument port of the endoscope control section. The neutralizer was injected and flushed through the endoscope channel from the suction port beside the endoscope light guiding connector, through the suction and biopsy channel and then the total volume of elution was collected from the distal end of the endoscope. The eluate was thoroughly mixed and sent to the laboratory of Shanghai Municipal Center for Disease Control and Prevention within 2 hours for culturing and colony counting (CFU/piece) (Figure 2).

(3) Disc brush sampling group (Group C): the endoscopes after reprocessed were sampled as the procedure below: a blunt head of a sterile disposable disc brush was inserted into the instrument port, and the instrument channel was brushed until the brush end completely exited the instrument channel outlet of the distal end. The upper part of the brush was cut off (2 cm) using sterile scissors, then the clipped brush was put into a sterile bottle for testing. 50 ml of neutralizer was extracted with a sterile syringe and injected into the instrument port, then the total volume of elution was collected from the distal end of the endoscope into the same bottle used for the testing of the clipped brush. The eluate was thoroughly mixed with the brush inside the sterile bottle and sent to the laboratory of Shanghai Municipal Center for Disease Control and Prevention within 2 hours for culturing and colony counting (CFU/piece) (Figure 3).

2.3. Colony Counts. In reference to the "Hygienic standard for disinfection in hospitals GB 15982-2012". The test solution was mixed thoroughly with a vortex mixer, inoculated with 1 ml of the mixed eluate in to two plates respectively, 20 ml of the molten nutrient agar medium cooled to 40°C–45°C was poured into each plate, and cultured in an incubator at 35°C for 48 hours, then the number of colonies (CFU/piece) were counted. The remaining eluate (48 ml) was filtered under sterile conditions using a filter membrane (0.45 μm). The inoculate filtered membrane was placed on a solidified nutrient agar

Table 1: Comparison of bacterial colony counts by different sampling methods (continuous sampling).

Sampling method	Max	Min	H	P
Biopsy channel (Group A)	1	0		
Entire channel (Group B)	1	0		
Disc brush (Group C)	21	0		
			2.657	0.265

Table 2: Comparison of bacterial colony counts by different sampling methods (intermittent sampling).

Sampling method	Max	Min	H	P
Biopsy channel (Group A)	1	0		
Entire channel (Group B)	36	0		
Disc brush (Group C)	30	0		
			5.626	0.060

plate and cultured in an incubator at 35°C for 48 hours, the number of colonies were counted.

2.4. Results Criteria. The cultured results of the samples were recorded. When the filter membrane method is in countable, the total number of colonies (CFU/piece) = m (CFU/plate) × 50. When the filter membrane method is countable, the total number of colonies (CFU/piece) = m (CFU/plate) + mf (CFU/filter membrane). In the formula, "m" is the average number of colonies on two parallel plates, and "mf" is the number of colonies on the filter membrane. When the colony number of the three methods are 0 CFU/, <1 CFU/ is used to represent the colony number of the three methods. Among them, the result was confirmed as negative if the number of bacterial colonies in the culture results was <1 CFU/piece, and the result was confirmed as positive if ≥1 CFU/piece.

2.5. Statistical Methods. The data was analyzed using SPSS 20.0. The statistical methods used are chi-square test for counting data and independent sample Kruskal-Walis test for measurement data.

Table 3: Comparison of bacterial colony counts group by different sampling methods (continuous sampling).

Sampling method	Colony count group	
	<1 (n, %)	≥1 (n, %)
Biopsy channel (Group A)	11 (91.7)	1 (8.3)
Entire channel (Group B)	8 (66.7)	4 (33.3)
Disc brush (Group C)	8 (66.7)	4 (33.3)
Total	27 (75)	9 (25)

Table 4: Comparison of colony counts group by different sampling methods (intermittent sampling).

Sampling method	Colony counts group	
	<1 (n, %)	≥1 (n, %)
Biopsy channel (Group A)	11 (91.7)	1 (8.3)
Entire channel (Group B)	7 (58.3)	5 (41.7)
Disc brush (Group C)	6 (50)	6 (50)
Total	24 (66.7)	12 (33.3)

3. Results

In this study, a total of 12 flexible endoscopes were collected using continuous sampling approach, with colony counts ranging from 0 to 21 CFU/piece. The detection rate of bacteria in the disc brush group (33.3%), and the entire channel group (33.3%) was higher than that of the biopsy channel group (8.3%). Among the 12 endoscopes sampled with intermittent approach, with colony counts ranging from 0 to 36 CFU/piece, the detection rate of bacteria from high to low was the disc brush group (50%), the entire channel group (41.7%), and the biopsy channel group (8.3%). It showed that the detection rate of bacteria for either the disc brush group or the entire channel group was higher than the biopsy channel group (Tables 1–4).

4. Discussion

4.1. Microbiology Culture Is the Gold Standard for Quality Control of Gastrointestinal Endoscope Cleaning and Disinfection. Gastrointestinal endoscopy is an important minimal invasive diagnosis and treatment method for gastrointestinal tract, pancreaticobiliary, and other diseases. It has complex and delicate structure and is difficult to thoroughly reprocess. In recent years, there have been many reports of endoscopy related healthcare associated infections with complex influencing factors. As a reusable medical device that directly contacts with the mucosa of a patient's organs, high-level disinfection, or sterilization must be achieved before use. However, studies have shown that even if the endoscope and accessories are treated strictly in accordance with guideline recommended for cleaning and disinfection methods, endoscopic associated infections are still possible to happen [8], so we believe that no matter the method of cleaning and disinfection, the effect must be verified to ensure the disinfection effect as well as patients' safety. At present, microbiology culture is an important way to evaluate the quality of the endoscope cleaning and disinfection [9].

4.2. There Are No Clear Standards and Operating Specifications Regarding Sampling Methods for Endoscope Microbiology Culture. A prospective study of the disinfection effect of a duodenoscope after disinfection and drying in one hospital found that the positive rate was 5–15.5% [10]. A study in Korea found that the positive rate for the treated duodenoscope was 37.2% [11]. Riberiro et al. from Brazil sampled the high-level disinfected endoscopes from 37 healthcare institutions in Minas Gerais, and found that the contamination rate of air-water channel of gastroscope was as high as 70% [12]. It was reported that currently the microbiological sampling methods of endoscopy are focus on antegrade and retrograde way, and the microbial positive rate is higher for the latter one [13–15]. It can be seen that there are wide differences between the results of gastrointestinal endoscopic microbiological cultures from

different countries. Different sampling methods will lead to the difference of microbiological culture results, and the false negative results will affect the reliability of the microbiology culture result. Thus, scientific and practical microbiological sampling methods are critical to ensure the quality of flexible endoscope reprocessing.

4.3. Positive Detection Rate Can Be Increased by the Disc Brush and the Entire Channel Sampling Method. In the endoscopic microbiology sampling method of our study, the entire channel group sampling was to inject the neutralizer from the instrument port beside the endoscope light guiding connector, and seal the suction port, the air/water injection port and the instrument port of the endoscope control section with the washing joint, and then collect the elution from the distal end of the endoscope. The path taken by the instrument channel sampling group is the same as the brush sampling group. The single biopsy channel was lacking the sampling of the suction channel compared with the entire channel group. However, the disc brush sampling method can brush and wash the inner surface of the endoscope lumen when compared with the simple flushing method of washing off the attachment from the inner surface of lumen. It has been shown that brush sampling method could scrub the endoscope channel and remove separately the new and old contaminants from endoscope channel [16]. It can be seen that the entire channel sampling method is more comprehensive, avoiding the limitations of the other two methods, and the disc brush sampling method could fully scrub the inner wall of the lumen, loosening and washing off the attachments, which might more effectively improve the detection rate of bacteria of endoscopes, but it may also require more staff cooperation between operators. The details, such as the type of brush, type of endoscope, the frequency of brushing practice etc. may need further research in the future [17].

4.4. Establishing Standard Microbiological Culture Sampling Method Is the Basis for Improving the Accuracy of Endoscopic Reprocessing Monitor Results. The results of this study shows that the positive rates of the entire channel sampling method and the disc brush sampling method are much higher than the conventional sampling method; whether, by continuous or intermittent approach. The conventional sampling method has been applied in China for nearly 15 years since 2004, and it will continue to be performed by healthcare practitioners in the future. There have been many international guidelines regarding to the endoscopic microbiology sampling, but the lack of well-defined illustrated operational procedures for specific sampling methods is likely to result in denormalization of microbiological sampling and reduced evaluability of results. Therefore, in the future, we can improve the sampling method for endoscopic microbiology culture through continuous research. At the same time, since the positive and negative judgments of the cultured results have no aligned international standard [6], the results of the colony counts in this experiment are grouped according to <1 and ≥ 1, thus it only represent the detection rate of bacteria instead of whether it is safe to use the endoscopes.

5. Conclusion

It was found in this study that both the entire channel sampling and the disc brush sampling method have higher bacterial positive detection rate than the conventional biopsy channel sampling method, which further indicates that the endoscopic sampling method being implemented currently needs further improvement. At the same time, this study also has certain limitations. This study is a single-center study. Meanwhile, the samples we used came from the gastrointestinal endoscopes used in daily clinical practice. The original bioburdens of the endoscope were not under control. Larger sample size and multi-center sites as well as endoscopic simulation models combined with laboratory experiments are needed in the future. Validation and comparison under standard condition could better increase the reliability and scientificity of the study.

Data Availability

The data used to support the findings of this study are included within the article.

Disclosure

The first author is Su Ma, the co-first author is Lili Feng, the co-first author is Wenxia Ding.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Kinetic of Adhesion of *S. epidermidis* with Different EPS Production on Ti6Al4V Surfaces

Miguel Ángel Pacha-Olivenza,[1,2,3] **Abraham Rodríguez-Cano**,[4] **M. Luisa González-Martín**,[2,3,4] **and Amparo M. Gallardo-Moreno**[2,3,4]

[1]*Department of Biomedical Sciences, Faculty of Medicine, University of Extremadura, Avda de Elvas s/n, Badajoz 06006, Spain*
[2]*Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Badajoz, Spain*
[3]*University Institute of Biosanitary Research of Extremadura (INUBE), Badajoz 06006, Spain*
[4]*Department of Applied Physics, Faculty of Science, University of Extremadura, Avda de Elvas s/n, Badajoz 06006, Spain*

Correspondence should be addressed to Amparo M. Gallardo-Moreno; amparogm@unex.es

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Controlling initial bacterial adhesion is essential to prevent biofilm formation and implant-related infection. The search for surface coatings that prevent initial adhesion is a powerful strategy to obtain implants that are more resistant to infection. Tracking the progression of adhesion on surfaces from the beginning of the interaction between bacteria and the surface provides a deeper understanding of the initial adhesion behavior. To this purpose, we have studied the progression over time of bacterial adhesion from a laminar flow of a bacterial suspension, using a modified Robbins device (MRD). Comparing with other laminar flow devices, such as the parallel plate flow chamber, MRD allows the use of diverse substrata under the same controlled flow conditions simultaneously. Two different surfaces of Ti6Al4V and two strains *of Staphylococcus epidermidis* with different exopolymer production were tested. In addition, the modified Robbins device was examined for its convenience and suitability for the purpose of this study. Results were analyzed according to a pseudofirst order kinetic. The values of the parameters obtained from this model make it possible to discriminate the adhesive behavior of surfaces and bacteria. One of the fitting parameters depends on the bacterial strain and the other only on the surface properties of the substrate.

1. Introduction

Orthopedic replacements help increase life expectancy and improve health conditions, especially in elderly patients. The progress in implant design is constant. Better mechanical properties and biocompatible surfaces make the devices more reliable. However, implant-associated infections are an unavoidable risk. Open fractures, contamination of the operating room or the patient's own skin or distal infections favor the presence of bacteria in the implant area [1]. In such a case, the likelihood of infection is greater if abiotic material is present than if no foreign material is involved. In fact, Elek and Conen [2] demonstrated in volunteers that local infections can be achieved with a bacterial concentration 10,000 times lower in the presence than in the absence of a synthetic material.

Colonization of implants by bacteria is a complex issue not fully understood. It is described that planktonic bacteria that get close enough to a material, due to their own motility or Brownian motion, can feel the surface and begin to land on the material. First long-range and then short-range forces, as van der Waals, electrical, hydrophobic, steric, and acid-base interactions drive the initial fixation of bacteria to the surface. Once on the surface, bond aging and biochemical interactions begin to play an important role through the polymeric structures present in the bacterial surface, strengthening the position of bacteria on the surface [3]. It is noteworthy that the adhesion of bacteria to surfaces triggers metabolic changes that make different the behavior of planktonic and sessile bacteria [4–6]. For some bacteria, the ability to produce exopolymeric substances (EPS) when they are on a surface is important. EPS sticks bacteria together and to the surface, helping the formation of biofilms. Biofilm provides an environment where bacteria are protected against external aggressions of the immune system or antibiotics. Its structure allows for some flow of nutrients and antibiotics,

but as bacteria within the biofilm can be maintained with different metabolic activities, the effectiveness of antibiotics against bacteria in the biofilm decreases [7, 8]. Virulence factors of bacteria are diverse. In the case of the most frequent microorganisms involved in early orthopedic infections, *Staphylococcus aureus* and *Staphylococcus epidermidis*, their virulence factors are different. While *S. aureus* produces a variety of toxins and adherence factors, the main virulence factor for *S. epidermidis* comes from its ability to produce EPS to adhere to biomaterials and form biofilms [9–13].

Avoiding the infectious process in the presence of a biofilm is a difficult challenge due to the stable and strong fixation to the surfaces that the biofilm provides to the bacteria. Since the elimination of biofilms is problematic, a plausible method to avoid infections is to circumvent the formation of biofilms by acting on the first interactions between planktonic bacteria and the surface. If the surface is modified to reduce the initial adhesion of bacteria, the appearance of biofilms can be expected to decrease [14]. Therefore, it is of primary interest to obtain information on the behavior of the adhesion process during the first period of exposure of the surface to bacteria.

The density of bacteria adhered during the initial phase is expected to be related to the physical-chemical interactions between the surface and the bacteria, like what happens with abiotic colloidal particles [15]. Several models have been developed to predict the behavior of this initial process, from the most schematic, assimilating bacteria with hard and bare particles to a more elaborate analysis considering them as soft particles with protuberances [16]. Other studies relate the bacteria of a culture with a mixed system of different colloids, as they have shown that subpopulations in the same bacterial culture may have different adhesion behavior due to the different degree of superficial heterogeneity between the bacteria of that same culture [17]. However, a holistic perspective of the process is given by monitoring the temporal variation in bacterial coverage on the surface [3]. Parameters related to the kinetics of adhesion can provide relevant information on the colonization process. Among them, the bacteria-substrate affinity, defined as the rate at which bacteria adhere to the surface during the first instants of contact between the material and the bacterial suspension [18]. Several authors have analyzed the initial affinity between bacteria and substrates and, in many cases, this information provided a good clue for predicting the ability of a given material to resist bacterial colonization [18–23].

There are various experimental approaches to obtaining this information. However, an overall assessment of these interactions is best obtained when suspended bacteria flow in a laminar regime over the surface [24]. There are many studies on the initial adhesion of bacteria to surfaces using different flow chamber designs. There are flow chambers that allow direct observation of the adhesion process on transparent or translucent or highly reflective materials [18, 25–33]. For other materials, samples must be removed from the chamber to stain bacteria for observation [34, 35].

Titanium and its alloys are used for orthopedic implants as they meet many of the requirements to allow their use in bone replacement. Ti6Al4V improves the resistance of commercially pure titanium increasing the elastic modulus, the maximum tensile strength, the fatigue strength, and the corrosion resistance [36]. In a previous publication, we presented a new process for the functionalization by cross-linked aminosilanization of Ti6Al4V that improves its biocompatibility [37]. In that study, we found that the coating developed protects Ti6Al4V against bacterial adhesion under static conditions. However, more relevant information on the initial interaction between the surface and the bacteria can be obtained if the adhesion takes place under flow. For this purpose we will follow the adhesion process on this material for two strains of *S. epidermidis* which that are expected to produce different biofilms due to their different EPS production capacity.

2. Materials and Methods

Unless otherwise stated, all reagents were purchased from Panreac Quimica S.A.U., Barcelona, Spain. Deionized water used in this study was obtained by purification with a Milli-Q Direct water system (Millipore, Madrid, Spain).

2.1. Substrates. Ti6Al4V disks (ELI grade 23) 25 mm in diameter and 2 mm in thickness were obtained from DKSH Ltd (Switzerland). According to the manufacturer, this is the chemical composition (wt. %) of the bars from which disks were obtained: Al (6.2–6.1%), V (3.99–4.06%), Fe (0.083–0.082%), C (0.01–0.02%), N (0.016–0.014%), H (0.003%), O (0.07–0.06%) and Ti (balance). Manufacturer also specifies these mechanical properties: tensile strength: 890/920 MPa, yield strength 0.2%: 810/845 MPa, elongation: 19/20% and reduction of area: 49/52%. Samples of Ti6Al4V were abraded with silicon papers (Buehler P320, 300 rpm, 2 min), mechanically polished with diamond paste (9 μm, 150 rpm, MetaDi Fluid, 10 min), and with colloidal silica (150 rpm, MasterMet2, 10 min). Then, disks were repeatedly rinsed and sonicated in deionized water (Milli-Q system), acetone and finally ethanol for periods of 10 min each. At the end, samples were dried at 40°C for 30 min and stored under vacuum for 12 h.

Disks of Ti6Al4V treated (T) were prepared from non treated (NT) disks subjected to an aminosilanization procedure, according to a methodology previously described [37]. In short, polished disks were subjected to chemical oxidation by treatment in a piranha solution (98% H_2SO_4 and 30% aqueous H_2O_2, 10 mL disk^{-1}) at 45°C for 30 min. Samples, denoted as NT were not subjected to any further treatment. A second set of samples, referred as "treated" were then immersed into a closed crystallizer containing a 1 M solution (10 mL disk^{-1}) of 3-(aminopropyl)trimethoxysilane (APTMS) in wet toluene ([H_2O] $\sim 8 \times 10^{-2}$ M). The reaction mixture was kept with stirring at 100 rpm, heated at 65°C until optimal coverage as inferred from the ninhydrin assay (usually 6 h). The disks were rinsed under flowing anhydrous toluene, then sonicated twice in the same solvent (30 mL disk^{-1}) for 10 min each, and dried under air. Disks were thermally cross-linked at 120°C during 24 h, then immersed in phosphate buffered solution (PBS, 20 mL disk^{-1}) at room temperature under stirring (700 rpm) for 48 h. Finally, disks were rinsed with distilled water and dried under a stream of Ar.

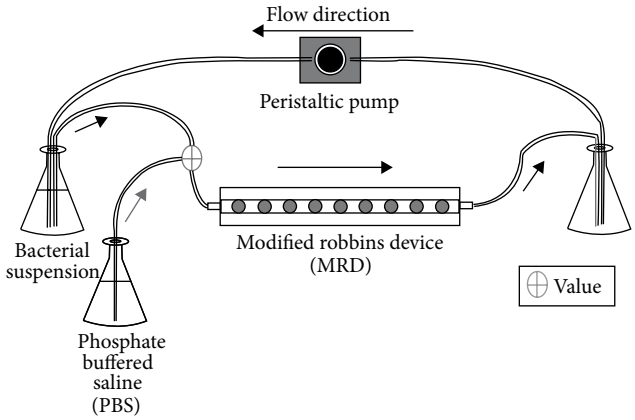

Figure 1: Scheme of the experimental setup for the adhesion experiments using a modified Robbins device (MRD).

All samples were treated together in order to minimize the heterogeneity. Consequently, experimental uncertainty in respect to hydrophobicity is low. Measured water contact angle on NT and T surfaces were $48 \pm 2°$ and $83 \pm 2°$, respectively [37].

2.2. Bacterial Strains and Culture. The strains used in the testwere *S. epidermidis* ATCC35983 (*S. epidermidis*3), medium EPS producer, and *S. epidermidis* ATCC35984 (*S. epidermidis*4) high EPS producer. These strains differ in their capacity of segregating polysaccharide intercellular adhesin when they are in a growth media [38, 39]. The strains were stored at −80°C in porous beds (Microbank, Pro-Lab Diagnostics, USA). Cultures were obtained from blood agar plates where bacteria from the frozen stock were inoculated and incubated at 37°C. From these cultures, tubes of 4 mL of Trypticase Soy Broth (TSB) (BBL, Becton Dickinson, USA) were incubated for 10 h and maintained at 37°C. Then 25 μL of this preculture was used again to inoculate 50 mL flasks of TSB at 37°C for 14 h. This time was enough to guarantee that strains were at the end of the exponential phase of growth, as it was checked with their growth curves that had been previously carried out. The bacteria were then harvested by centrifugation for 5 min at 1000 rpm (Sorvall TC6, Dulont, USA) and washed three times with 0.15 M phosphate buffered saline, PBS, ($0.87\,g{\cdot}L^{-1}$ of K_2HPO_4, $0.68\,g{\cdot}L^{-1}$of KH_2PO_4 and $8.76\,g{\cdot}L^{-1}$ of NaCl) pre-conditioned at 37°C. Afterwards, bacteria were re-suspended in a volume of PBS enough for the whole adhesion experiment, at a concentration of 3×10^8 bacteria mL^{-1}. Along the test, this stock suspension was actively stirred to ensure a constant bacterial concentration.

2.3. Bacterial Adhesion. Adhesion experiments were carried under flow using a Modified Robbin Device (MRD). Figure 1 shows a scheme/diagram of the MRD with the experimental setup. This flow chamber allows testing several samples under the same bacterial suspension. The MRD is a pipe of rectangular section, along which the samples are placed. The flow inside the MRD was kept at a constant rate of $2\,mL{\cdot}min^{-1}$. Under these conditions, flow was in laminar regime, the Reynolds number was 1.64, the wall shear rate was $0.97\,s^{-1}$, and the shear stress was $6.8\ 10^{-4}\,N{\cdot}m^{-1}$. Samples were exposed to the bacterial flow using supports screwed through one of the walls of the MRD, ensuring they were perfectly leveled to avoid any turbulence of the flowing liquid. The MRD used allowed testing nine different samples simultaneously. Any support with a sample can be extracted from the device at any time and replaced with other support with a dummy surface attached.

Once all the samples were in their position in the MRD, PBS was let to flow for 15 min. Then, the flow was gently switched to the bacterial suspension, and after 5, 10, 15, 20, 30, 40, 60, and 90 min, one sample at each time was removed. Extracted samples were passed by a PBS solution and then dried in a sterile environment. Afterwards, bacteria on the surface were stained with SYTO9 (Invitrogen SA, Spain), observed by epifluorescence microscopy and automatically counted with the software NIS-Elements BR 4.10 (Nikon Instruments INC., USA). Counting on each sample was done on at least 9 places randomly selected. All the experiments were done at 37°C, and repeated at least three times with independent cultures. For each of the times tested, the samples were removed from different positions in the MRD in each of repetition of the experiment.

Due to the complexity of the experimental procedure, reproducibility tests of the whole experiment were carried out. One aspect that needs consideration is the distance of each sample to the flow entrance, since the samples are in line in the same direction than flow. Some studies have found dependence on this distance, but positive or negative depending on the liquid velocity [40]. Another aspect that needs attention deals with the procedure of extraction and measurement of the number of bacteria on each surface. When samples are removed from MRD, their surface passes from liquid to air. That passage can remove from the surface bacteria not enough attached to surmount the force acting on them by surface tension. In the control experiments, all the samples were under the same bacterial flow during the same time. Then, samples in even positions in the MRD were removed and replaced by dummy samples. Next, an air bubble was injected through the inner channel of the MRD that implies the passage of two air-liquid interfaces over the surfaces that could sweep bacteria from the samples, simulating the force suffered in extraction. Afterwards, samples in the odd positions were removed. Finally, quantification of adhered bacteria on each surface was done as described previously. These experiments were triplicated, and the number of adhered bacteria per unit area were comprised between $(43 \pm 2) \times 10^4$ bacteria$\cdot cm^{-2}$ and $(45 \pm 3) \times 10^4$ bacteria$\cdot cm^{-2}$ in odd positions and $(42 \pm 3) \times 10^4$ bacteria$\cdot cm^{-2}$ and $(46 \pm 3) \times 10^4$ bacteria$\cdot cm^{-2}$ in even positions. This test ensures that our results are reproducible within an uncertainty level lower than 10%.

2.4. Statistical Analyses. The data are expressed as means ± standard deviation. Differences between the means were determined by a one-way analysis of variance (ANOVA) after confirmation of data normality using IBM SPSS Statistics v19 (IBM company, New York, USA). Statistical significance was accepted for $p < 0.05$ after comparing the mean values by the Tukey HSD test.

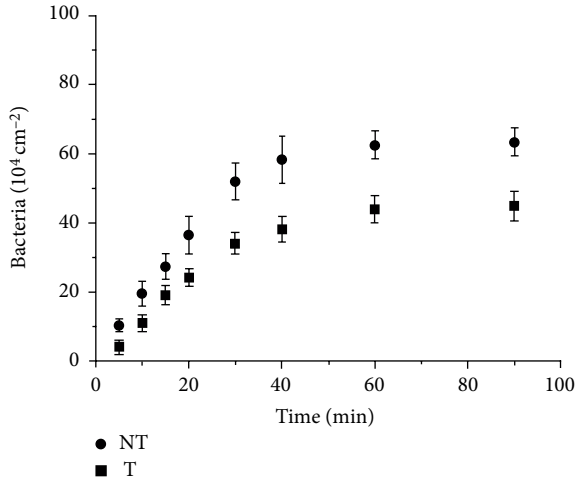

Figure 2: Temporal evolution of *S. epidermidis*3 adhesion on treated (T) and nontreated (NT) surfaces.

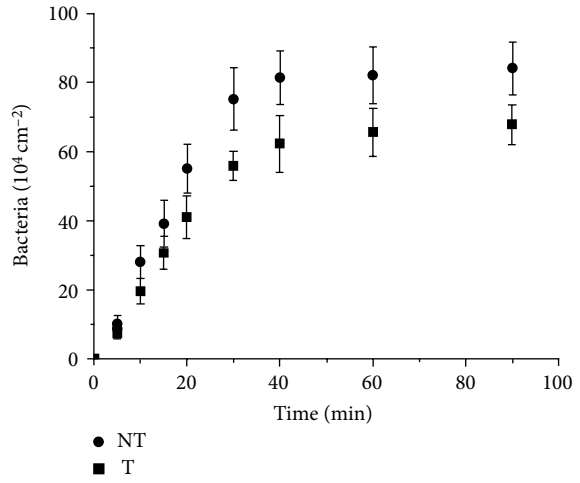

Figure 3: Temporal evolution of *S. epidermidis*4 adhesion on treated (T) and nontreated (NT) surfaces.

3. Results and Discussion

Once a material is put in contact with a bacterial suspension, microorganisms begin the colonization of the surface. This process happens for stagnant as well as flowing bacterial suspensions. Figures 2 and 3 show the time dependence of the surface density of retained bacteria on the treated (T) and the nontreated (NT) surfaces, for *S. epidermidis*3 and *S. epidermidis*4, respectively. In all cases, the adhesion rate is depending on the time elapsed since the beginning of the flow, decreasing as the coverage of bacteria increases. This behavior can be related to a gradual occupation of surface positions by bacteria, masking an area that could account up to thirty times its geometrical section, as for abiotic particles [41], making difficult for newcomers the access to the surface. Both strains adhere more on the NT than on the T surfaces, but, for each surface condition, the retention of *S. epidermidis*4 is higher than for *S. epidermidis*3. Figure 4 presents various images of typical areas on treated (T) and nontreated (NT) surfaces as function of the contact time to better visualize the attachment of bacteria under flow conditions.

Modelling bacteria as colloidal abiotic particles provides successful approaches to analyze bacteria-surface interplay. Visualization of samples after 90 min of adhesion (images not shown) exhibit bacteria well dispersed on the surface. Assuming microorganisms as colloidal particles, bacterial retention could fit a model in which the number of adhered bacteria depends on the available positions on the surface, with no contribution of bacterial co-adhesion. This model, schematically $A + B \rightarrow AB$, is associated to a pseudofirst order kinetic that assumes the rate of surface coverage as proportional to the free positions on the surface:

$$\frac{d\theta(t)}{dt} = k(1 - \theta(t)) \tag{1}$$

being $\theta(t)$ the surface coverage, defined as $\theta(t) = n(t)/n_\infty$; $n(t)$ the bacterial density on the surface at time t, and n_∞ its limit value at very long time. k is a proportionality constant that should be related to the retention energy of bacteria on the surface. Integration of Equation (1) leads to

$$n(t) = n_\infty\left(1 - e^{-kt}\right). \tag{2}$$

Table 1 includes the values of n_∞ and k obtained for each of the conditions studied. For each surface, n_∞ is lower for *S. epidermidis*3 than for *S. epidermidis*4. Also, for each bacterial strain, n_∞ is lower on the treated surface than on the nontreated one, being this reduction equals to 29% for *S. epidermidis*3 and 19% for *S. epidermidis*4.

From Equation (2) it is also possible to evaluate the interaction between bacteria and substrate at the very initial stages of the temporal evolution of the adhesion, or bacterium-substrate affinity, j_o, according to:

$$j_o = \lim_{t\to 0} j(t) = \lim_{t\to 0} \frac{dn(t)}{dt}. \tag{3}$$

Table 1 shows the values of j_o obtained. Affinity results points at the same behavior than shown by n_∞. For any of the surface finishes, affinity is higher for *S. epidermidis*4 than for *S. epidermidis*3. Also, for each of the bacterial strains, affinity is lower for T than for NT surfaces, ranging from $(1.7 \pm 0.3) \times 10^4\,\text{cm}^{-2}\cdot\text{min}^{-1}$ to $(4.1 \pm 0.6) \times 10^4\,\text{cm}^{-2}\cdot\text{min}^{-1}$. This similar behavior is also observed in other systems. Progress of the adhesion of S. *epidermidis*4 and other bacterial strains on surfaces of stainless steel and Ti6Al4V that were submitted to different treatments, show also a direct correlation between the affinity, j_o, and the limit value of the number of adhered bacteria, n_∞ [21–23].

Assumption of bacteria as colloidal particles is accepted as a model to obtain information of the real behavior of these microorganisms on surfaces. Sjollema et al. [42] evaluated the deposition rate of a flowing suspension of colloidal particles, j_{SL}, according to the Smoluchowski-Levich approach given by

$$j_{SL} = \frac{DC}{a} Sh, \tag{4}$$

where C is the concentration of particles of radio a in the suspension, D is the Stoke-Einstein diffusion coefficient and Sh the Sherwood number [43]

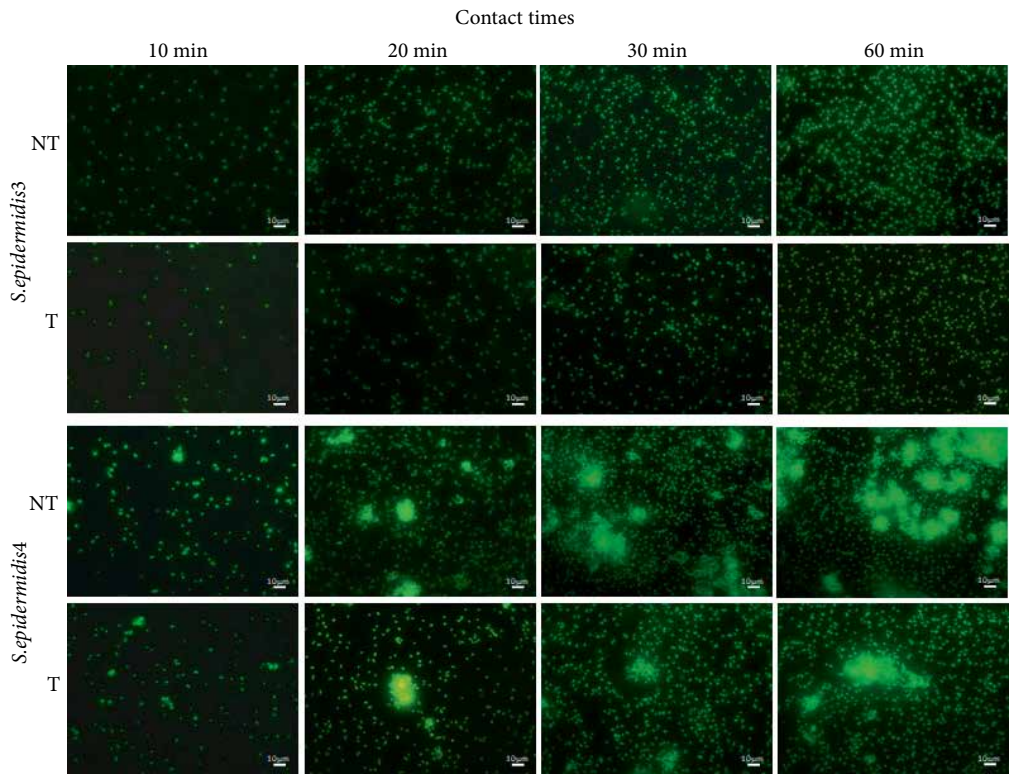

Figure 4: Fluorescence imagesof typical areas on treated (T) and nontreated (NT) surfaces as function of the contact time under flow conditions for *S. epidermidis*3 and *S. epidermidis*4.

Table 1: Fitted parameters from Equations (2) and (3), n_∞, k, and j_o, to the treated (T) and nontreated (NT) surfaces for *S. epidermidis*3 and *S. epidermidis*4.

Surface-strain	n_∞ (bact $cm^{-2} \times 10^4$)	k (min^{-1})	j_o (bact $cm^{-2} min^{-1} \times 10^4$)
NT-*S. epidermidis*3	69 ± 3	0.039 ± 0.004	2.7 ± 0.4
T-*S. epidermidis*3	49 ± 3	0.034 ± 0.003	1.7 ± 0.3
NT-*S. epidermidis*4	91 ± 6	0.045 ± 0.004	4.1 ± 0.6
T-*S. epidermidis*4	74 ± 4	0.040 ± 0.005	3.0 ± 0.5

$$Sh = \frac{1}{\Gamma(4/3)} \left(\frac{2bP_e}{9x} \right)^{1/3}, \quad (5)$$

being x the distance from the flow inlet and b the half-depth of the MRD, and P_e the Pèclet number given by $P_e = 3va^3/2b^2D$, being v the fluid velocity. For our experimental conditions, P_e equals to 6.2×10^{-5}, and Sh equals to 7.9×10^{-3}.

Then, from Equation 4, the theoretical upper limit for the initial deposition rate, j_{SL}, obtained is 1.24×10^4 bacteria·cm^{-2}·min^{-1}, lower than any of the j_o experimental values obtained (Table 1). Other authors also found discrepancies between the theoretical and the experimental deposition rates for bacterial suspensions, who related to the failure on the fulfillment of conditions for the application of the Smoluchowski-Levich approach, due to the radius and/or to possible appendages of bacteria protruding from the surface [18, 44].

Nevertheless, it can be expected that differences in surface properties between strains imply also different behavior against surfaces, so it should be expected that different behavior not only depends on the strain but also on the alloy surface. The properties of the surface modify the deposition rate also for abiotic particles, as found by Dabroś and van de Ven for polystyrene particles on cover glass slides, where the deposition rate was found to be dependent on the cleaning method of the slides and conditioned to interactions at separations smaller than 10 nm [41].

To find as much clear as possible the dependence on both factors of adhesion, namely surface characteristics of the strain and surface characteristic of the material, it is of interest following the evolution with time of the actual experimental surface coverage, $\theta_e(t) = n(t)/n_{90}$. In this way, the fitting parameters in a pseudofirst order kinetic equation can be used to modulate the exponential behavior of the coverage. Then, taking the maximum experimental bacterial density n_{90} as a reference for each experiment, the bacterial retention could be described as modified pseudofirst order kinetics, according to

$$1 - \theta_e(t) = ge^{-k_e t}. \quad (6)$$

This equation is similar to Equation (2), but being g and k_e adjustable parameters instead of n_∞ and k. The values obtained for g and k_e are listed in Table 2. In all cases, k_e is larger than k and g is larger than one. It is remarkable that it appears that g and k_e are uncoupled, each of them from the surface characteristics and the microorganisms, respectively. The obtained values of g depend only on the bacterial strain, in agreement

Table 2: Fitted parameters from Equations (6) g k_e, and j_{oe}, to the treated (T) and nontreated (NT) surfaces for *S. epidermidis*3 and *S. epidermidis*4.

Sample-strain	n_{90} (bact $cm^{-2} \times 10^4$)	g	k_e (min^{-1})	j_{oe} (bact $cm^{-2} min^{-1} \times 10^4$)
NT-*S. epidermidis*3	63 ± 3	1.58 ± 0.15	0.075 ± 0.014	7 ± 2
T-*S. epidermidis*3	45 ± 2	1.57 ± 0.03	0.067 ± 0.003	4.7 ± 0.5
NT-*S. epidermidis*4	85 ± 6	1.29 ± 0.12	0.074 ± 0.008	8 ± 2
T-*S. epidermidis*4	68 ± 3	1.30 ± 0.10	0.064 ± 0.005	5.7 ± 1.1

with the proposal that discrepancies from the colloidal model are related to the bacterial characteristics [44]. Values of g are higher for *S. epidermidis*3 than for *S. epidermidis*4, that suggest worse initial effectiveness in adhesion of *S. epidermidis*3 than *S. epidermidis*4, irrespective of the surface finish. Surface charge is an important factor in the adhesive behavior of microorganisms. We have previously measured the zeta potential of both strains in PBS. The values were $-(16 \pm 8)$ mV for *S. epidermidis*3 (unpublished result) and $-(6 \pm 2)$ mV for *S. epidermidis*4 [21]. Nevertheless, despite a slightly higher zeta potential for *S. epidermidis*3, experimental uncertainties do not ensure that the electrical properties make a difference in the behavior of the two strains. However, EPS production appears to be a discriminating characteristic between both strains. *S. epidermidis*3 is a moderately EPS-producing strain in contrast with *S. epidermidis*4 that produces EPS in a large extent. This is reflected in their surface characteristics. An AFM study showed that *S. epidermidis*4 is fully covered by a slime layer with higher adhesion to the tip than the slime for *S. epidermidis*3 which is only partially slime-covered [39]. Song et al. [45] studied the vibration of some bacterial cells adhered on glass under static and flow conditions. They found that the vibrational amplitude of *S. epidermidis*3 is significantly higher than for *S. epidermidis*4, being the higher vibrational amplitudes associated to smaller spring constants. This behavior suggests a firmer and better localized adhesion of *S. epidermidis*4, being more closely related to the irreversible adhesion process given by $A + B \rightarrow AB$ given by a g value equal to 1.

On the other hand, the exponential parameter k_e relates the surface availability and the rate of the coverage. This means that for a given coverage, as much larger the k_e parameter is, the larger the rate of surface occupation will be. For a given occupation of the surface, it reflects the capability of the surface of the material to attract new particles that in turn is related to the properties of the material. In the present study, the treatment applied to the surface of Ti6Al4V yields a k_e lower than for the original surface. That suggests that the coverage provided to samples protects in some extension the alloy material from bacterial colonization. That protection was observed in static experiments [37]. The amino groups introduced in surface, carrying positive charge, could favor adhesion of negatively charged bacteria. However, this attractive contribution is not enough to surpass its capability to conceal the affinity of bacteria for the surface of Ti6Al4V, enriched in OH groups by the piranha solutions, previous to the silanization process. Interestingly, this protection against bacteria resists flow conditions.

Using Equation (3), the initial slope, j_{oe}, of the process described by Equation (6) can be evaluated as $j_{oe} = n_{90}.g.k_e$. This new parameter is listed in Table 2. In all cases, j_{oe} is higher than j_o. The higher value of j_{oe} is consequence of the short difference between n_∞ and n_{90}, despite the larger differences in the time associated to each of these two bacterial densities. Interestingly, it appears that, within the experimental uncertainty, j_{oe} is independent of the bacterial strain, no matter the large differences of the coverages after 90 minutes, n_{90}, of both strains. This result suggests that the j_{oe} parameter provides a clearer gauge than k_e for the adhesive behavior of the surfaces against the *S. epidermidis* strains tested.

4. Conclusions

The use of a modified Robbins device allows the monitoring of bacterial adhesion on Ti6Al4V with different surface treatment. Bacteria tested differ on their ability to produce EPS. From the dynamic adhesion information provided using the Robbins device, an analysis based on a pseudofirst order kinetics allows to discriminate the behavior of each surface and each strain on the surfaces. Since the calculated parameter g can be related to the strain, the j_{oe} parameter reports only from the adhesion characteristics of the Ti6Al4V surface. Additional studies will allow us to extend this analysis to other groups of bacteria and/or surface modifications.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Research Article

Prevalence of Tongue Cleaning Using a Toothbrush: A Questionnaire Survey in Fukui Prefecture, Japan

Shinpei Matsuda,[1] **Takehisa Saito**,[2] **Hisato Yoshida**,[1] **Hitoshi Yoshimura**,[1] **and Kazuo Sano**[1,3]

[1]*Department of Dentistry and Oral Surgery, Unit of Sensory and Locomotor Medicine, Division of Medicine, Faculty of Medical Sciences, University of Fukui, Fukui, Japan*
[2]*Department of Otorhinolaryngology, Head and Neck Surgery, Unit of Sensory and Locomotor Medicine, Division of Medicine, Faculty of Medical Sciences, University of Fukui, Fukui, Japan*
[3]*Oral Care Support Center, Fukui Dental Association, Japan*

Correspondence should be addressed to Shinpei Matsuda; shinpeim@u-fukui.ac.jp

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Objective. The aim of this study was to investigate the tongue cleaning habits using toothbrushes among outpatients of the University of Fukui Hospital and a private hospital in Fukui Prefecture. *Methods.* We administered a questionnaire survey to volunteers detailing tongue cleaning habits using toothbrushes. The content of the questions in this survey were as follows: gender, age, frequency of tongue cleaning, portion of tongue cleaning, and purposes of tongue cleaning. *Results.* We had 1,014 volunteers of various ages participating in this study. Regarding the frequency of tongue cleaning, 187 (18.4%) of all participants replied, "Every day", and 346 (34.1%) replied, "Sometimes". Regarding tongue cleaning of the 533 participants with active tongue cleaning habits, 242 (45.4%) participants replied, "The center of the dorsum of the tongue", and 274 (51.4%) replied, "The entire tongue". When analyzing the purpose of tongue cleaning, 346 (64.9%) participants replied, "To remove the tongue stain", 192 (36.0%) participants replied, "To remove the tongue coating", and 240 (45.0%) participants replied, "To manage halitosis". *Conclusions.* This study clarified that a wide range of age groups in the nonhospitalized general public practiced tongue cleaning habits using a toothbrush for various purposes.

1. Introduction

Oral care brings various benefits. In 1999, Yoneyama et al. reported that oral care could decrease the risk of pneumonia in the institutionalized elderly [1]. In institutionalized elderly, the importance of oral care, including tongue cleaning has received attention, and several studies have reported on the topic. Izumi et al. reported that oral care with tongue cleaning is important for preventing aspiration pneumonia, because it could improve coughing ability [2]. Recently, the relationship between tongue coating and food type were clarified, and its result suggested that tongue cleaning should be performed with consideration for the food type [3].

In hospitalized patients, infectious complications, such as ventilator-associated pneumonia (VAP) and surgical site infection, sometimes lead to critical conditions [4–6]. Some studies have suggested that oral care, including tongue cleaning, could reduce those complications, and that oral care improving the quality of patient care was economically viable [4–6]. Also, Tajima et al. reported that tongue cleaning was also useful for elders fed with a feeding tube because it could decrease the number of microbes on the tongue surface [7].

As the aging of the population progresses in Japan, the importance of oral care is widely known not only to institutionalized elderly or hospitalized patients, but also to the nonhospitalized general public. With the increasing interest in oral care, attention has also been focused on tongue cleaning. Tongue cleaning has been practiced for centuries all over the world with tongue scrapers made from various materials, such as silver, gold, copper, tin, brass, and plastic [8]. Tongue cleaning is usually performed to remove tongue coating, and it has been considered to contribute to oral bacterial control and halitosis management [9–13]. Halitosis is not usually associated with acute oral or systemic infections, but it may be associated with chronic periodontal disease or the chronic action of bacteria in tongue coating [14, 15]. In addition, a

Table 1: Frequency of tongue cleaning using a toothbrush.

Frequency Age	Gender	Every day *n* (%)	Sometimes *n* (%)	Never *n* (%)	Total
15–19		48 (20.8)	79 (34.2)	104 (45.0)	231 (22.8)
	Male	6 (11.1)	16 (29.6)	32 (59.3)	
	Female	42 (23.7)	63 (35.6)	72 (40.7)	
20–29		23 (15.9)	65 (44.8)	57 (39.3)	145 (14.3)
	Male	10 (13.5)	30 (40.5)	34 (45.9)	
	Female	13 (18.3)	35 (49.3)	23 (32.4)	
30–39		25 (24.3)	35 (34.0)	43 (41.7)	103 (10.2)
	Male	4 (13.3)	8 (26.7)	18 (60.0)	
	Female	21 (28.8)	27 (37.0)	25 (34.2)	
40–49		29 (18.8)	51 (33.1)	74 (48.1)	154 (15.2)
	Male	8 (19.5)	11 (26.8)	22 (53.7)	
	Female	21 (18.6)	40 (35.4)	52 (46.0)	
50–59		17 (18.3)	21 (22.6)	55 (59.1)	93 (9.2)
	Male	1 (3.1)	7 (21.9)	24 (75.0)	
	Female	16 (26.2)	14 (23.0)	31 (50.8)	
60–69		25 (18.5)	43 (31.9)	67 (49.6)	135 (13.3)
	Male	14 (25.0)	13 (23.2)	29 (51.8)	
	Female	11 (13.9)	30 (38.0)	38 (48.1)	
70–79		13 (12.6)	40 (38.8)	50 (48.5)	103 (10.2)
	Male	3 (7.7)	13 (33.3)	23 (59.0)	
	Female	10 (15.6)	27 (42.2)	27 (42.2)	
80<		7 (14.0)	12 (24.0)	31 (62.0)	50 (4.9)
	Male	0 (0)	3 (23.1)	10 (76.9)	
	Female	7 (18.9)	9 (24.3)	21 (56.8)	
	Total	187 (18.4)	346 (34.1)	481 (47.4)	1014 (100)

recent study reported that continuous tongue brushing improved subjective taste, such as sweet, salty, sour, and bitter [16, 17]. Seerangaiyan et al. suggested that tongue cleaning could increase the salt taste intensity and help individuals adhere to the World Health Organization recommendations on dietary salt intake [17]. Furthermore, tongue cleaning might improve gingival inflammation and digestive power [18, 19]. In contrast to those studies, one of our previous studies using confocal laser scanning microscopy in combination with a filter-paper disc method suggested that excessive tongue cleaning might lead to a decreased number of both fungiform papillae and taste buds associated with taste sensation [20]. Based on the results of these studies, we considered that moderate or mild mechanical tongue cleaning may be acceptable and yield some positive effects, but excessive tongue cleaning may affect taste sensation. The chorda tympani nerve innervates the taste buds of the fungiform papillae in the anterior two-thirds of the tongue [21]. Also, the distribution of fungiform papillae innervated by the chorda tympani nerve is substantial in the tip and midlateral regions of the tongue [22, 23]. Therefore, we considered that brushing the entire tongue, including the midlateral and tip regions, might affect the fungiform papillae associated with taste sensation [20].

Recently, many specific tongue cleaning tools have been produced and sold [24, 25]. On the other hand, a recent study on tongue cleaning reported that a toothbrush was the most common tongue cleaning tool [26]. However, most studies about tongue cleaning do not include the toothbrush tongue cleaning practice despite of the difference in mechanical cleaning force applied to the tongue. Additionally, there have been no reports about portion and purposes of the tongue cleaned using a toothbrush. Therefore, we considered that it was important to investigate the actual prevalence, portion, and purposes of tongue cleaning using a toothbrush.

Medical professionals should know the actual conditions of tongue cleaning using a toothbrush. Then, medical professionals should disseminate and give medical advice about oral care based on this information. However, there has been no large-scale survey about the effects of tongue cleaning using toothbrushes or information about the cleaned portion of the tongue. The aim of this study was to investigate the habits of tongue cleaning using toothbrushes among nonhospitalized people in Fukui Prefecture, Japan.

2. Materials and Methods

2.1. Ethical Approval. This study was an observational study, and approved by the Institutional Research Board (Ethics Committee of University of Fukui, Faculty of Medical Sciences; No. 20150088).

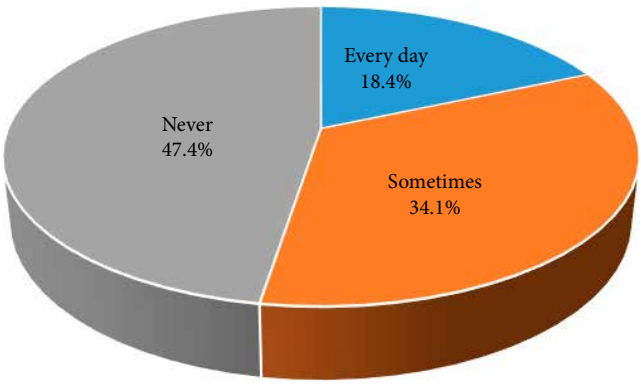

Figure 1: Frequency of tongue cleaning using a toothbrush.

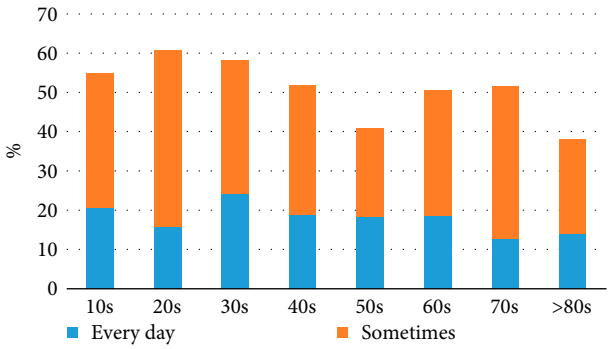

Figure 2: Percentage of participants who had tongue cleaning habits.

2.2. Participants. A questionnaire survey of tongue cleaning using a toothbrush was given to volunteers of various ages between 2015 and 2016. The participants were outpatients of the Department of Otorhinolaryngology, Head and Neck Surgery of University of Fukui Hospital and a private hospital in Fukui Prefecture, Japan. In addition, to eliminate bias, this questionnaire survey was not conducted by dental professionals, including dentists and oral hygienists, or in places related to dental treatment and oral surgery. To obtain appropriate answers for questionnaires, only populations aged 15 or over participated in this study. Participants with incomplete responses to the questionnaire were excluded.

2.3. Questionnaires. The content of the questions in this survey was as follows: (1) gender, (2) age, (3) frequency of tongue cleaning, (4) portion of tongue cleaning, and (5) purposes of the tongue cleaning. The ages were divided into "10s", "20s", "30s", "40s", "50s", "60s", "70s", and "80s and over". The frequency of tongue cleaning was divided into "Every day", "Sometimes", and "Never". In this questionnaire survey, "Never" meant that participants had no experience with the tongue cleaning using a toothbrush for tongue cleaning. The portion of the tongue cleaning detailed the following three cases: "The center of the dorsum of the tongue", "The entire tongue", and "The others". The following three purposes of tongue cleaning were detailed: "To remove tongue stain", "To remove tongue coating", and "To manage halitosis". Also, multiple answers were permitted only for the question about purposes of the tongue cleaning.

Table 2: Portion of tongue cleaning using a toothbrush.

Portion	Gender	Center* n (%)	Entire** n (%)	Others*** n (%)	Total (%)
	Male	62 (42.2)	81 (55.1)	4 (2.7)	147 (27.6)
	Female	180 (46.6)	193 (50.0)	13 (3.4)	386 (72.4)
		242 (45.4)	274 (51.4)	17 (3.2)	533 (100)

*The center of the dorsum of the tongue. **The entire tongue. ***The others.

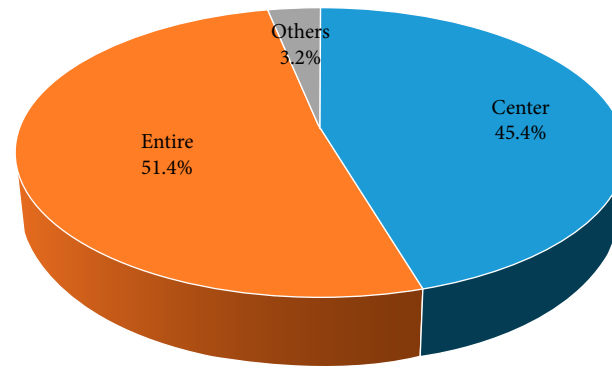

Figure 3: Portion of tongue cleaning using a toothbrush.

Table 3: Purposes of tongue cleaning using a toothbrush.

Purpose	Gender	To remove tongue stain n (%)	To remove tongue coating n (%)	To manage halitosis n (%)
	Male	95 (64.6)	54 (36.7)	64 (43.5)
	Female	251 (65.0)	138 (35.8)	176 (45.6)
	Total (%)	346 (64.9)	192 (36.0)	240 (45.0)

2.4. Statistical Analyses. The relationships between frequency of tongue cleaning and each age group, between portion of tongue cleaning and gender, and between purposes of tongue cleaning and gender were analyzed statistically. In frequency analysis, the male and female participant groups were analyzed separately. Statistical analyses were performed using the IBM SPSS Statistics version 25 statistical software (IBM Japan Ltd., Tokyo, Japan). A chi-squared test was used to assess the statistically significant relationship. The value of $p < 0.05$ was considered statistically significant.

3. Results

There were 1,014 participants of various ages who completed our questionnaires for this study. The participants consisted of 339 males (33.4%) and 675 females (66.6%). The mean age and standard deviation of those participants was 42.7 ± 21.8 years. The youngest subject was 15 years old, and the oldest subject

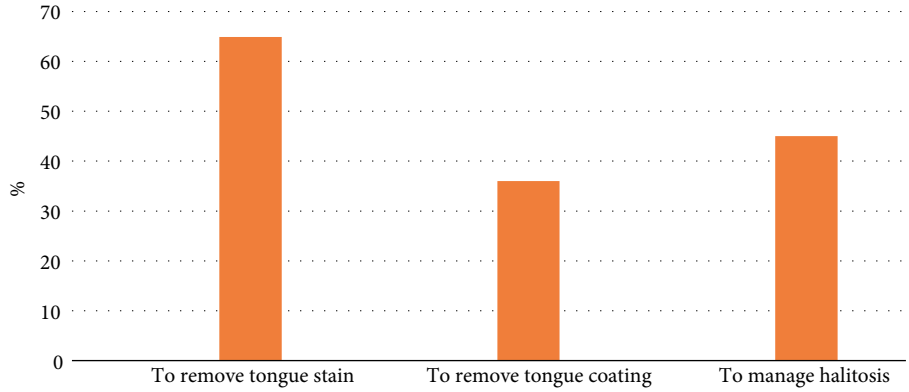

Figure 4: Purpose of tongue cleaning using a toothbrush.

was 92 years old. The group of "10s" (231 participants, 22.8%) was the largest group, and the group of "80s and over" (50 participants, 4.9%) was the smallest group. The average number and standard deviation of each age group was 126.8 ± 53.7. Regarding the frequency of tongue cleaning, 187 (18.4%) of all participants replied, "Every day", 346 (34.1%) replied "Sometimes", and 481 (47.4%) replied "Never" (Table 1, Figure 1). Forty-six (13.6%) male participants replied, "Every day", 101 (29.8%) replied "Sometimes", and 192 (56.6%) replied, "Never". One hundred forty-one (20.9%) female participants replied, "Every day", 245 (36.3%) replied, "Sometimes", and 289 (42.8%) replied "Never". When considering both aspects of the frequency of tongue cleaning and age groups, the highest percentage of participants that replied "Every day" was the 30s group (24.3%), and the lowest was the 70s group (12.6%). In regard to male participants, the highest percentage of respondents that replied, "Every day" was the 60s group (25.0%), and the lowest was the over 80s group (0%). In female participants, the highest percentage of respondents that answered "Every day" was the 30s group (28.8%), and the lowest was the 60s group (13.9%). When summing the percentages of participants who replied, "Every day" and "Sometimes", namely, percentage of participants who practiced tongue cleaning habits regardless of the frequency, the highest was the 20s group (60.7%), and the lowest was the over 80s group (38.0%) (Figure 2). There was no statistically significant relationship between the frequency of tongue cleaning and age groups in neither male nor female participants ($p = 0.083$ vs. $p = 0.077$, chi-squared tests). In regard to the portion of tongue cleaning, 242 (45.4%) of 533 participants with tongue cleaning habits replied, "The center of the dorsum of the tongue", and 274 (51.4%) replied, "The entire tongue" (Table 2, Figure 3). There was no statistically significant relationship between portion of tongue cleaning and gender ($p = 0.564$, chi-squared tests). In regard to the purposes of tongue cleaning, 346 (64.9%) of 533 participants replied "To remove the tongue stain," 192 (36.0%) of them replied "To remove the tongue coating", and 240 (45.0%) of them replied "To manage halitosis" (Table 3, Figure 4). The analysis of gender differences in the purpose of tongue cleaning based on the sum of participants that replied "Everyday" and "Sometimes" showed as follows: 95 (64.6%) of 147 male participants and 251 (65.0%) of 386 female participants replied "To remove the tongue stain", 54 (36.7%) male participants and 138 (35.8%) female participants replied "To remove the tongue coating", and 64 (43.5%) male participants and 176 (45.6%) female participants replied "To manage halitosis." There was no statistically significant relationship between purposes of tongue cleaning and gender ($p = 0.944$, chi-squared tests).

4. Discussion

This study investigated the prevalence of tongue cleaning using a toothbrush through a relatively large-scale questionnaire survey administered to the nonhospitalized general public in Fukui Prefecture, Japan. Although the cleaning frequency was different among age groups, the results of this questionnaire survey clarified that 533 (52.6%) of 1,014 participants had tongue cleaning habits using toothbrushes. There was no statistically significant relationship between the frequency of tongue cleaning and age groups in both male and female participants. These results suggested that a wide range of age groups of the general public have had a great interest in tongue cleaning, indicating that the importance of oral care is widespread not only in medical professionals, but also the general public. In regard to the portion of tongue cleaning, the number of participants that replied, "The entire tongue" was slightly higher than the number of participants that replied, "The center of the dorsum of the tongue". Also, there was no statistically significant relationship between portion of tongue cleaning and gender. In regard to the purposes of tongue cleaning, the response "To remove the tongue stain" was most common. Interestingly, when analyzing the various percentages for the purposes of tongue cleaning in relation to the participants who had the tongue cleaning habits, regardless of the frequency, we observed close values between male and female participants for all three purposes, and there was no statistically significant relationship between purposes of tongue cleaning and gender. That result indicated that gender did not play a role in tongue cleaning purpose. However, it should be noted that multiple answers were permitted only for the question about purposes of the tongue cleaning. This study suggested that the general public cared about tongue cleanliness and halitosis, and tongue cleaning using a toothbrush was performed in several different ways for different purposes.

Kishi et al. conducted a questionnaire survey about tongue cleaning habits for 479 participants and reported that 37.0% of all participants replied that they practiced tongue cleaning [26]. Although it is widely known that there are special tools for brushing tongues, a toothbrush (81.4%) was the most common tongue cleaning tool [26]. However, there were no questions about the portion of the tongue cleaned by cleaning tools in their study [26]. In this study, a questionnaire survey associated with tongue cleaning using a toothbrush was conducted based on that report. Their survey was conducted at two public health centers that held monthly meetings for health promotion and at one dental hospital [26]. On the other hand, our survey was neither conducted by dental professionals, including dentists and oral hygienists, nor in places related to dental treatment and oral surgery for elimination of bias. Thus, we considered that the results of this questionnaire survey were reliable and beneficial. Because our questionnaires needed to be as simple as possible, we selected five questions, involving gender, age, frequency, portion, and purposes of the tongue cleaning, to clarify the summary of tongue cleaning habits.

The effects of mechanical tongue cleaning were examined in some basic bacteriological studies. Matsui et al. reported about a study using a disposable tongue cleaner equipped with a cleaner head composed of a urethane sponge and their study suggested that tongue cleaning reduced the amount of bacteria on the tongue [9]. Some literature has reported on the effect of tooth brushing accompanied with tongue cleaning compared to tooth brushing only, and the former had a significant effect on the reduction of halitosis and tongue coating [27, 28]. Furthermore, Bordas et al. concluded that mechanical tongue cleaning with or without chemical intervention, such as mouthwash, could reduce bacterial load on the tongue [28].

Mechanical tongue cleaning may have positive effects on oral bacterial load, halitosis management, and subjective taste [9–13, 16, 17]. In contrast to those reports, one of our previous studies suggested that excessive mechanical tongue cleaning might lead to damage of the gustatory receptors, and progression of this damage might have an association with the decreased number of taste buds that exist on the surface of the fungiform papillae [20]. Kullaa-Mikkonen et al. reported that fungiform papillae and taste buds are distributed over the dorsum of the tongue and that they are more common at the tip and on the edge than in the middle of the tongue [22]. Additionally, it is important that the tongue coating, including desquamated epithelial cells, food debris, bacteria, and salivary proteins, was usually found in the mid-distal part of the dorsum of the tongue [15, 29]. The results of these studies suggested that the tongue cleaning should not be performed at the tip and on the edge of the tongue for the purpose of tongue coating removal, although moderate cleaning of the center of the tongue is acceptable. Medical professionals should keep in mind that the oral cavity structures might be easily damaged, and excessive mechanical tongue cleaning may affect those delicate and sensitive anatomical structures, such as fungiform papillae and taste buds.

In this study, we investigated the prevalence of tongue cleaning using only a toothbrush. The limitations of this study were that this was a questionnaire survey and that there is no additional information about the clinical oral environment. Furthermore, to simplify the survey, we chose a selection-type questionnaire in items of frequency, portion, and purposes of the tongue cleaning. In addition, we should consider that there is dispersion in the number of the participants in both aspects of gender and ages in this study. We need to consider the possibility that the results in this study were influenced by characteristics of the Japanese culture and the tendency of Japanese individuals to prefer cleanliness.

With the increasing interest in oral care, prevalence of tongue cleaning and the market of cleaning tools will expand. Rickenbacher et al. reported that acceptance of the use of a tongue vacuum cleaner among children was higher than acceptance with a child's manual toothbrush [25]. However, there are few reports discussing the differences between tongue cleaning using a toothbrush and other mechanical tongue cleaning methods. Also, there are many types of toothbrushes depending on form and hardness. Therefore, we considered that the research on tongue brushing is inadequate at this time. Based on the results obtained from this research, further studies associated with tongue management methods, such as cleaning tools, frequency, degree, and teaching and assistance methods, should be carried out. These results will provide the general public with valuable information on suitable tongue management methods in the future.

5. Conclusions

This study found that a wide range of age groups in the nonhospitalized general public performed tongue cleaning using a toothbrush for various purposes. Further studies associated with tongue management methods will provide the general public with valuable tongue cleaning information in the future.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest and no sources of funding with regard to this manuscript.

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Research Article

The Change of Laboratory Tests Could Be Predictive Factors for Infection after McKeown Esophagogastrectomy

Chongxiang Chen,[1,2] Tianmeng Wen ,[3] and Qingyu Zhao [1]

[1]*Department of Intensive Care Unit, Sun Yat-Sen University Cancer Center, State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Guangzhou, 510060, China*
[2]*Guangzhou Institute of Respiratory Diseases, State Key Laboratory of Respiratory Disease, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, 510120, China*
[3]*School of Public Health, Sun Yat-Sen University, Guangzhou, Guangdong Province, China*

Correspondence should be addressed to Qingyu Zhao; zhaoqy163@163.com

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Aim. To analyze whether the change of laboratory tests (postoperative day 1 (POD1) minus pre-operation) could be predictive factors for postoperative infection in patients who have undergone McKeown esophagogastrectomy. *Methods.* We retrospectively investigated the clinical data of 358 patients who have undergone McKeown esophagogastrectomy, and divided them into infection and noninfection groups. SPSS 22.0 software was performed for data analysis. *Results.* In the two groups, smoking status (66.7% vs. 42.3%; $P = 0.014$), male gender (86.1% vs. 72.0%; $P < 0.001$), hoarseness (23.6% vs. 8.7%; $P < 0.001$), poor coughing ability (51.4% vs. 9.1%; $P < 0.001$), the change of WBC count ($5.59 \pm 4.75 \times 10^9$/L vs. $4.51 \pm 4.11 \times 10^9$/L; $P = 0.05$), the change of glucose (6.03 ± 3.97 g/L vs. 3.78 ± 3.18 g/L), the change of ALB (-12.83 ± 3.45 g/L vs. -10.69 ± 3.86 g/L), the change of CRE (0.17 ± 19.94 umol/L vs. -4.02 ± 15.40 umol/L, $P = 0.047$) were significantly different. These factors were assessed using logistic regression analysis, and factors with $P \leq 0.05$ in the univariate analysis were entered into multivariate analysis based on the forward stepwise (conditional) method. Poor coughing ability (odds ratio [OR], 11.034, 95% confidence interval [CI], 5.358–22.724), smoking status (OR, 4.218; 95% CI, 2.110–8.431), the change of WBC count (OR, 1.079; 95% CI, 1.000–1.164), the change of serum ALB level (OR, 0.849; 95% CI, 0.772–0.935), and the change of blood glucose levels (OR, 1.237; 95% CI, 1.117–1.371) were determined as independent risk factors for postoperative infection. We established a scoring system based on these 5 factors, and the area under the curve for this predictive model was 0.843 (range, 0.793–0.894); the sensitivity, specificity, and cut-off score were 70.8%, 85.3%, and 2.500, respectively. *Conclusion.* Poor coughing ability, smoking habit, the high change of WBC and blood glucose levels, and low change of serum ALB levels can be used to predict the occurrence of postoperative infections among patients who have undergone McKeown esophagogastrectomy.

1. Introduction

Up to now, Esophageal cancer is the sixth-most common cause of cancer-related death all around the world, and in developing countries, it is the fifth most frequent cause of deaths [1]. Furthermore, the incidence and mortality of patients with esophageal cancer in China were the highest globally in 2009 [2]. Surgery remains the standard treatment for resectable esophageal cancer. However, esophagogastrectomy is a complex procedure, with morbidity and mortality rates of 23%–50% and 2%–8%, respectively [3, 4].

Patients undergoing McKeown esophagogastrectomy are exposed to a higher risk of infection compared with those receiving other types of surgery. Moreover, patients with esophageal cancer are at a greater risk of antimicrobial exposure due to their impaired immunological functions and are also at an increased risk of infection with multidrug-resistant bacteria.

In this study, we assumed that the change of laboratory tests (laboratory tests within 24 h after surgery minus pre-operation) will be associated with the infections following McKeown esophagogastrectomy, and developed recommendations for clinicians treating patients with these risk factors.

2. Methods

2.1. Data Collection. We collected clinical data from 358 esophageal cancer patients (including 268 male and 90 female patients) who were admitted for McKeown esophagogastrectomy (right thoracotomy followed by laparotomy and cervical anastomosis) between July 2014 and October 2016 at Sun Yat-Sen University Cancer Center (SYSUCC). The RDD number for this study is RDDA2019001127. The average age of the patients was 60.55 ± 7.87 years. We divided the patients into the infection and noninfection groups according to the occurrence of postoperative infections, and then retrospectively assessed the baseline characteristics, clinical disease features, perioperative features, preoperative, and postoperative laboratory test results (including white blood cell [WBC], neutrophil, hemoglobin [HB], aspartate aminotransferase [AST], alanine aminotransferase [ALT], serum albumin [ALB], blood urea nitrogen [BUN], creatinine [CRE], blood glucose, C-reactive protein [CRP], and lactic acid levels) between the groups. All the postoperative day 1 (POD1) indicators were analyzed within 24 h after surgery. And we used POD1 indicators minus preoperative ones to calculate the change of laboratory tests.

2.2. Inclusion and Exclusion Criteria. The inclusion criteria were as follows: patients aged >18 years with esophageal cancer who underwent McKeown esophagogastrectomy and developed an infection during hospitalization. And those with infection prior to hospital admission were excluded from the study.

2.3. Statistical Analysis. Categorical variables were expressed as number and percentage, and continuous variables were expressed as mean ± standard deviation. Student's *t*-test was used to examine continuous variables, and the Chi-squared test or Fisher's exact test was used to assess categorical variables. Multi-variate analysis was performed to determine the predictors of postoperative infection, and the forward stepwise (conditional) method was used to identify factors to enter into the multivariate regression model. Receiver operating characteristic (ROC) curves were constructed to estimate the sensitivity, specificity, and the area under the curve (AUC) for various cutoff points of the relevant indicators. Statistical significance was set at $P \leq 0.05$, and all statistical analyses were computed using SPSS Version 22.0.

3. Results

3.1. Differences in the Baseline Characteristics. Table 1 describes the characteristics of the 72 patients (20.1%) with postoperative infection, from among the 358 patients who had undergone McKeown esophagogastrectomy in the present study. We compared the patients' baseline characteristics and clinical disease features between groups, and identified significant differences in smoking habits and gender between the two groups. The smoking habit frequency (66.7% vs. 42.3%; $P < 0.001$) and proportion of males (86.1% vs. 72.0%; $P = 0.014$) were greater in the infection group than in the noninfection group (Table 1 and Figure 1).

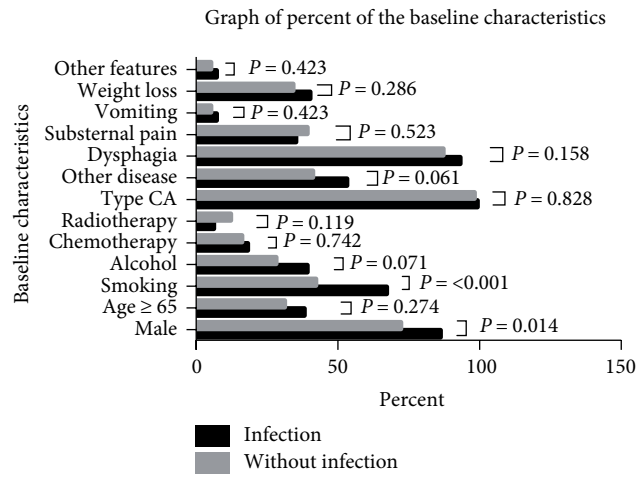

Figure 1: Baseline characteristics and clinical disease features between the infection group and noninfection group.

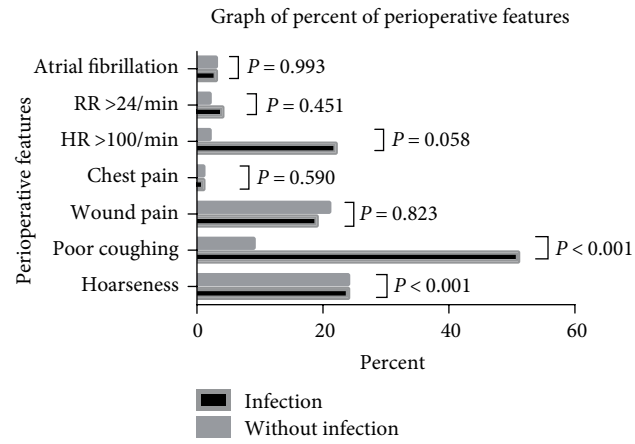

Figure 2: Difference in perioperative features among patients who underwent McKeon esophagogastrectomy.

3.2. Differences in the Perioperative Clinical Features. In the present study, the factors of hoarseness (23.6% vs. 8.7%; $P = 0.001$) and poor coughing ability (51.4% vs. 9.1%; $P < 0.001$) were significantly different between the groups; both were more frequent in the infection group. However, other perioperative clinical features, including wound pain, increased heart rate and respiratory rate, chest pain/chest distress, and atrial fibrillation, did not exhibit a significant difference (Table 2 and Figure 2).

3.3. Differences in Change of Laboratory Test Results. The results of the change of laboratory tests (POD1 minus pre-operation) were compared between the groups. The change of WBC count ($5.59 \pm 4.75 \times 10^9$/L vs. $4.47 \pm 4.14 \times 10^9$/L; $P = 0.048$), neutropil count ($7.00 \pm 4.63 \times 10^9$/L vs. $5.83 \pm 3.69 \times 10^9$/L; $P = 0.023$), glucose (6.03 ± 3.97 g/L vs. 3.78 ± 3.18 g/L; $P < 0.001$), ALB (−12.83 ± 3.45 g/L vs. −10.69 ± 3.86 g/L; $P < 0.001$), CRE (0.17 ± 19.94 umol/L vs. −4.02 ± 15.40 umol/L, $P = 0.047$) were greater in the infection group than in the noninfection group. None of the other change of laboratory test results showed significant differences (Table 3 and Figure 3).

Table 1: Baseline characteristics and clinical disease features between the infection group and noninfection group.

Outcome	Infection group (%)	Noninfection group (%)	χ^2	P value
Sum	72	286		
Gender			6.062	0.014*
Male	62 (86.1)	206 (72.0)		
Female	10 (13.9)	80 (28.0)		
Age			1.195	0.274
≥65	27 (37.5)	88 (30.8)		
<65	45 (62.5)	198 (69.2)		
Smoking habit			13.695	<0.001*
Yes	48 (66.7)	121 (42.3)		
No	24 (33.3)	165 (57.7)		
Alcohol consumption			3.254	0.071
Yes	28 (38.9)	80 (28.0)		
No	44 (61.1)	206 (72.0)		
Chemotherapy			0.108	0.742
Yes	13 (18.1)	47 (16.4)		
No	59 (81.9)	239 (83.6)		
Radiotherapy			2.431	0.119
Yes	4 (5.6)	34 (11.9)		
No	68 (94.4)	252 (88.1)		
Type of cancer			0.047	0.828
Squamous	71 (98.6)	281 (98.3)		
Others	1 (1.4)	5 (1.7)		
Other chronic disease			3.503	0.061
Yes	38 (52.8)	116 (40.6)		
No	34 (48.2)	170 (59.4)		
Dysphagia			1.995	0.158
Yes	67 (93.1)	249 (87.1)		
No	5 (6.9)	37 (12.9)		
Substernal pain			0.408	0.523
Yes	25 (34.7)	111 (38.8)		
No	47 (65.3)	175 (61.2)		
Acid regurgitation/vomiting			0.641	0.423
Yes	5 (6.9)	13 (4.5)		
No	67 (93.1)	273 (95.5)		
Weight loss			1.140	0.286
Yes	29 (40.3)	96 (33.6)		
No	43 (59.7)	190 (66.4)		
Other clinical features			0.641	0.423
Yes	5 (6.9)	13 (4.5)		
No	67 (93.1)	273 (95.5)		

*Statistically significant at $P \leq 0.05$.

3.4. Multivariate Analysis. Factors that were significant in the univariate analysis ($P < 0.05$) were included in the multivariate analysis. Accordingly, we assessed 5 factors, including poor coughing ability (odds ratio [OR], 11.034; 95% confidence interval CI, 5.358–22.724), smoking status (OR, 4.218; 95% CI, 2.110–8.431), the change of WBC count (OR, 1.079; 95% CI, 1.000–1.164), ALB level (OR, 0.849; 95% CI, 0.772–0.935), blood glucose level (OR, 1.237; 95% CI, 1.117–1.371), using multivariate regression; male gender and the other laboratory test results were not included (Table 4).

3.5. Development of a Scoring System to Predict Postoperative Infections. The AUC and cut-off point were 0.575 (range, 0.498–0.651) and 4.420×10^9/L for the change of WBC count, 0.725 (range, 0.660–0.790) and 4.355 mmol/L for the change of blood glucose level, and 0.658 (range, 0.590–0.727) and −11.900 mmol/L for the change of serum ALB level, respectively.

Patients with were assigned a score of 1 for each of the following factors: poor coughing ability, smoking habit, the change of WBC count and blood glucose levels greater than the cut-off values, and the change of ALB level lower than the

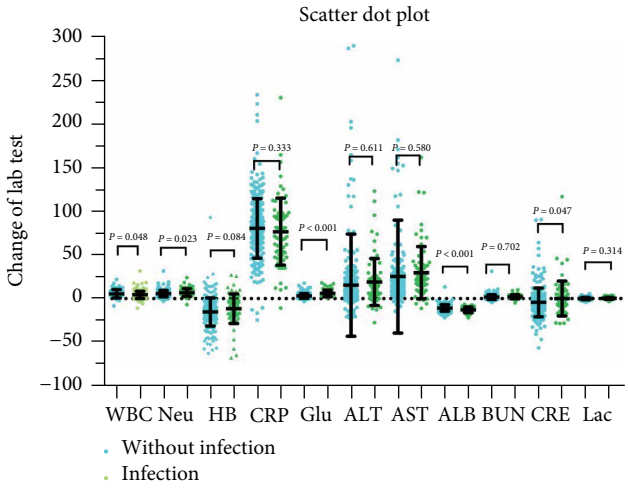

Figure 3: Scatter dot plot of the change of laboratory tests between two groups.

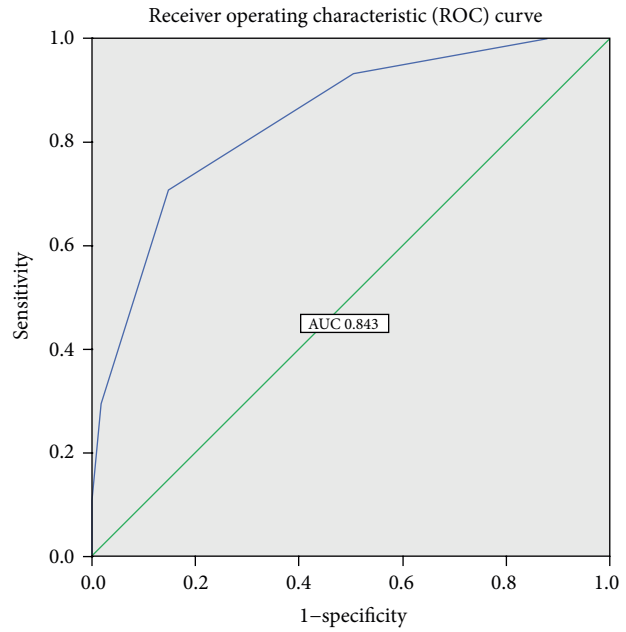

Figure 4: Receiver operating characteristic (ROC) curve of the scoring system.

cut-off value; patients who did not meet these requirements were assigned a score of 0 each.

The AUC of this predictive model was 0.843 (range, 0.793–0.894); the sensitivity, specificity, and cut-off score were 70.8%, 85.3%, and 2.500, respectively (Figure 4 and Table 5).

3.6. Pulmonary Complication through Clinical Diagnosis. The differences of changes of laboratory tests in the groups divided by pulmonary complication were showed in the supplementary data.

4. Discussion

Results of comparing the infection and noninfection groups in the present study indicated that poor coughing ability, smoking status, the change of WBC count, the change of ALB level, and the change of blood glucose level were independent risk factors for predicting postoperative infection in patients undergoing McKeown esophagogastrectomy.

According to our analysis, smoking acts as one of the independent risk factors for predicting postoperative infection. Liu et al. [5] reported that smoking history was one of the risk factors of postoperative lung infection. Moreover, the study conducted by Kinugasa et al. [6] showed that smoking habit was risk factors for postoperative pulmonary complications. Furtermore, the similar result shown in the study conducted by Ferguson et al. [7]. Smoking history could increase airway resistance, then cause numerous postoperative sputum thrombi. Consequently, the risk of pulmonary infection increases by impairment of the respiratory epithelium cilia structure, damaging to goblet cells, and weakening cilia movement.

The result of our study showed that the change of WBC count was associated with the postoperative infection. Sugita et al. [8] showed that the preoperative WBC count did not differ between infected and noninfected patients, although the POD1 WBC count was significantly higher in infected patients than in noninfected patients. The study conducted by Gomez et al. [9] also showed that the median WBC count was significantly greater in patients with infection than in those without infection during the first 10 postoperative days.

Furthermore, we found that the change of ALB level was an independent risk factor for postoperative infection in patients underwent McKeown esophagogastrectomy. The study conducted by Yin et al. [10] showed that low serum albumin was independently associated with the surgical site infections (SSI). Moreover, Zhao et al. [11] demonstrated that ALB level <35 g/L was an independent risk factor for postoperative infectious complications in patients with hepatocellular cancer. In addition, Yuwen et al. [12] showed that an ALB level of <35 g/L was associated with an increased risk of SSI in patients after orthopedic operations.

Lastly, our study showed that the change of blood glucose level was an independent risk factor for predicting infection. Similar to our findings, Vriesendorp et al. [13] indicated that the POD1 blood glucose level in esophageal cancer patients after esophagectomy was only associated with the length of hospitalization. Moreover, Ng et al. [14] showed that the change in the target glucose control in diabetic patients was independently associated with an increase in SSI. Another study conducted by Ambiru et al. [15] demonstrated that the SSI rates were correlated with the hyperglycemia following surgery.

In the scoring system of our study, the continuous variables changed to categorical variables through the cut-off value (higher than cut-off value is number A, lower than the cut-off value is number B), and added value for each factor. All the categorical variables used assigns 20% for each factor in the total score because it was useful and easy in clinical practice.

I think our study is quite novelty. On the one hand, our study talked about specific disease (esophageal cancer), and undergoing specific kind of operation (McKeown

Table 2: Difference in perioperative features among patients who underwent McKeon esophagogastrectomy.

Outcome	Infection group (%)	Noninfection group (%)	Statistic (χ^2 or T value)	P value
Total no. of patients	72	286		
Hoarseness			12.282	<0.001*
Yes	17 (23.6)	25 (8.7)		
No	55 (76.4)	261 (91.3)		
Poor coughing ability			70.967	<0.001*
Yes	37 (51.4)	26 (9.1)		
No	35 (48.6)	260 (90.9)		
Wound pain			0.050	0.823
Yes	14 (19.4)	59 (20.6)		
No	58 (80.6)	227 (79.4)		
Chest pain/chest distress			0.290	0.590
Yes	1 (1.4)	2 (0.7)		
No	71 (98.6)	284 (99.3)		
Heart rate			3.586	0.058
>100/min	16 (22.2)	7 (2.4)		
≤100/min	56 (77.8)	279 (97.6)		
Respiratory rate			0.567	0.451
>24/min	3 (4.2)	7 (2.4)		
≤24/min	69 (95.8)	279 (97.6)		
Atrial fibrillation			0.000	0.993
Yes	2 (2.8)	8 (2.8)		
No	70 (97.2)	278 (97.2)		
MAP	90.20 ± 9.83	89.83 ± 10.33	−0.281	0.779

*Statistically significant at $P \leq 0.05$. MAP: Mean artery pressure

Table 3: Difference in the change laboratory test results between the infection and noninfection group (POD1 minus Pre-McKeown esophagogastrectomy).

	Infection group	Noninfection group	T value	P value
WBC ($\times 10^9$/L)	5.59 ± 4.75	4.47 ± 4.14	−1.987	0.048*
Neutrophils ($\times 10^9$/L)	7.00 ± 4.63	5.83 ± 3.69	−2.276	0.023*
HB (g/L)	−11.55 ± 17.01	−15.29 ± 16.21	−1.735	0.084
Serum ALB (g/L)	−12.83 ± 3.45	−10.69 ± 3.86	4.295	<0.001*
ALT (IU/L)	19.18 ± 26.88	15.57 ± 58.71	−0.509	0.611
AST (IU/L)	29.72 ± 29.96	25.38 ± 64.76	−0.554	0.580
BUN (mmol/L)	2.06 ± 2.28	1.88 ± 2.77	−0.514	0.702
CRE (μmol/L)	0.17 ± 19.94	−4.02 ± 15.40	−1.996	0.047*
Glucose (mmol/L)	6.03 ± 3.97	3.78 ± 3.18	−4.525	<0.001*
CRP (mg/L)	76.97 ± 38.48	81.21 ± 32.93	0.968	0.333
Lactic acid (mmol/L)	0.34 ± 1.32	0.18 ± 1.21	−1.007	0.314

*Statistically significant at $P \leq 0.05$. WBC, white blood cell; HB, hemoglobin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALB, serum albumin; BUN, blood urea nitrogen; CRE, creatinine; CRP, C-reactive protein.

esophagogastrectomy); on the other hand, in our study, we used the changes of these laboratory tests different from other studies.

The limit of our study is that it is a single center retrospective research, and the study population comprised only Asian participants. The patient number enrolled in our study was relatively small, so some risk factor, such as alcohol consumption were not included in the independent risk factors. Probably, in the future study, we could get more data to conduct a tendentious matching analysis to better identify the risk factors. Moreover, we do not have postoperative pulmonary function tests in our clinical practice; we need prospective study to address this problem for better diagnosing the respiratory complication, which is one of the major complications of esophagectomy [16, 17]. Our study did not compare the minimally invasive esophagectomy with open invasive esophagectomy, some studies showed that minimally invasive esophagectomy had lower incidence of postoperative infection than open invasive esophagectomy [18–20]. In addition, other factors should be contained in further study, such as operation time, intraoperative bleeding, the application of antacids, and so on.

Table 4: Multivariate logistic regression analysis of the risk factors for infections after McKeown esophagogastrectomy.

Variate	Univariate analysis			Multivariate analysis		
	P	OR	95% CI	*P*	OR	95% CI
Gender	0.016	0.415	(0.203–0.850)			
Smoking habit	<0.001*	2.727	(1.584–4.695)	<0.001*	4.218	(2.110–8.431)
Poor coughing ability	<0.001*	10.571	(5.725–19.520)	<0.001*	11.034	(5.358–22.724)
Hoarseness	<0.001*	3.227	(1.633–6.378)			
Change of ALB level	<0.001*	0.855	(0.795–0.921)	=0.001*	0.849	(0.772–0.935)
Change of WBC	0.051	1.059	(1.000–1.123)	=0.050	1.079	(1.000–1.164)
Change of neu	0.027	1.073	(1.008–1.143)			
Change of CRE	0.059	1.013	(1.000–1.027)			
Change of glucose	<0.001*	1.283	(1.177–1.398)	<0.001*	1.237	(1.117–1.371)

*Statistically significant at $P \leq 0.05$. Change of ALB: serum ALB level within 24 h after surgery minus pre-operation. Change of WBC level: serum WBC level within 24 h after surgery minus pre-operation. Change of neutropils: serum neutropils level within 24 h after surgery minus pre-operation. Change of CRE: serum CRE level within 24 h after surgery minus pre-operation. Change of glucose: blood glucose level within 24 h after surgery minus pre-operation. Factors were entered into multivariate regression using the forward stepwise (conditional) approach ($P \leq 0.05$).

Table 5: Receiver operating characteristics of the independent risk factors and the scoring system.

Factors	AUC (95% CI)	*P* value	Cut-off	Sensitivity (%)	Specificity (%)
Change of WBC level	0.575 (0.498–0.651)	0.039*	4.420	61.1	53.8
Change of glucose level	0.725 (0.660–0.790)	<0.001*	4.355	62.5	74.8
Change of ALB level	0.658 (0.590–0.727)	<0.001*	−11.900	59.7	67.1
Scoring system	0.843 (0.793–0.894)	<0.001*	2.5	70.8%	85.3%

*Statistically significant at $P \leq 0.05$. Change of ALB: serum ALB level within 24 h after surgery minus pre-operation. Change of WBC level: serum WBC level within 24 h after surgery minus pre-operation. Change of glucose: blood glucose level within 24 h after surgery minus pre-operation. With regard to the scoring system, patients were assigned a score of 1 for each of the following factors: poor coughing ability, smoking habit, change of WBC count and blood glucose levels greater than the cut-off values, and change of ALB level lower than the cut-off value, whereas patients who did not meet these requirements were assigned a score of 0 each.

5. Conclusion

Patients are exposed to high risks of predicting postoperative infection after McKeown esophagogastrectomy, although poor coughing ability, smoking habit, the change of WBC count, the change of ALB level, and the change of blood glucose level may be as independent risk factors for postoperative infections in these patients. At last, we used a scoring system comprising these 5 factors, and observed that the AUC of this predictive model was 0.843 (range, 0.793–0.894), whereas the sensitivity, specificity, and cut-off score were 70.8%, 85.3%, and 2.500, respectively.

Data Availability

The datasets used and/or analyzed in the current study are available from the corresponding author upon request.

Consent

All authors have agreed to the publication of this manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Chongxiang Chen designed the study. Chongxiang Chen, Qingyu Zhao designed the search strategy and performed the search. Chongxiang Chen, and Tianmeng Wen performed abstract screening, full text screening, data extraction, and risk of bias assessment. Chongxiang Chen and Qingyu Zhao drafted the manuscript. All authors revised the manuscript, as well as reading and approving the final manuscript.

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Supplementary Materials

The supplementary data includes a table of the difference of the changes of laboratory tests divided by clinical diagnosis pulmonary complication. (*Supplementery Materials*)

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Research Article

Bloodstream Infection and Its Clinical Characteristics and Relevant Factors Associated with Interventional Therapy in a Large Tertiary Hospital: A Six Years Surveillance Study

Yanling Bai,[1] Zhigang Zheng,[2] Mingmei Du,[1] Hongwu Yao,[1] Yunxi Liu ,[1] and Jijiang Suo [1]

[1]*Department of Infection Management and Disease Control, Chinese PLA General Hospital, Fuxing Road No. 28, Beijing 100853, China*
[2]*The First Outpatient Department of General Logistics Department, Fuxing Road No. 22, Beijing 100842, China*

Correspondence should be addressed to Yunxi Liu; 1425628298@qq.com and Jijiang Suo; sjj301@qq.com

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Background. Interventional therapy has been widely used in the medical field as its advantages of minimally invasive, safe and quick recovery. Bloodstream infection (BSI) is the most common Healthcare-Associated Infections (HAIs) after interventional therapy, but there are few reports about it. This study intends to analyze the clinical characteristics and relevant factors of BSI after six years of interventional therapy in a large tertiary teaching hospital, in order to provide guidances for the prevention and control of BSI after interventional operations. *Methods*. The case information of patients with BSI after interventional therapy from 2013 to 2018 were collected through the "real-time monitoring system of healthcare-associated infections". All BSI was determined by the infection control full-time staff and clinicians. Questionnaires were designed to review case by case and register the relevant patient information into a database. A total of 18 relevant factors were counted. Statistical software was used for analysis. *Results*. 174 cases of BSI occurred in 25401 patients, the incidence was 0.69%, and BSI accounted for 50% of all infected sites. Gram-positive bacteria accounted for 56.05%, coagulase-negative Staphylococcus was the main infectious bacteria. Relevant risk factor analysis showed that hepatocellular carcinoma, had undergone surgery, biliary complications, prophylactic antibiotic, replacement of antibiotics, number of interventional operations, days of prophylactic antibiotic use were the related risk factors associated with BSI ($P < 0.05$). Multivariate analysis showed that days of prophylactic antibiotic use (OR = 1.586, $P < 0.05$) and replacement of antibiotics (OR = 13.349, $P < 0.05$) were the main risk factors associated with the development of BSI. *Conclusions*. BSI is the main infection site after interventional surgery. For patients with the risk factors as hepatocellular carcinoma/biliary complications/had undergone surgery, etc., the time of prophylactic antibiotic use can be prolonged properly before interventional surgery, and selection of single antibiotic appropriate for use could significantly aid preventive measures to avoid occurrence of BSI.

1. Introduction

Interventional therapy, with its advantages of minimally invasive, safe and rapid recovery, has been rapidly developed since its application in medical treatment in the 1970s, especially in the diagnosis and treatment of hepatocellular carcinoma (HCC) and hepatobiliary diseases [1]. Under the condition of no exposure to the lesion, it uses catheters, guide wires and other devices to carry out minimal trauma treatment for some lesions under the guidance of imaging devices (X-ray, ultrasound, CT, MRI) [2]. However, many invasive operations such as puncture, intubation, and drug injection need to be performed during interventional operation, and extended length of stay in hospital environment after operation provides a way for pathogenic bacteria to invade. Therefore, Healthcare-associated Infections (HAIs) is the most common complication [3–5]. According to the research, the incidence of Bloodstream infection (BSI) in patients with HCC after angiography is 4.0% [6], and after embolization is 2.7% [7]. Therefore, it is important to find out the risky factors of BSI in time and give effective treatment as soon as possible for the rehabilitation of patients after operation.

To our knowledge, this type of study focused on the analysis of the infection situation of specific treatment methods

for a certain disease. There were few and old studies on specific infection sites (such as BSI) [4–8]. This study analyzed the clinical characteristics and relevant risk factors of BSI after interventional therapy in a large hospital from 2013 to 2018, aiming at providing guidances for prevention and control of BSI.

2. Materials and Methods

2.1. Materials. From January 1, 2013 to December 31, 2018, a total of 25401 inpatients in a tertiary hospital in Beijing in China were collected. 174 of them suffered from BSI. According to inclusion exclusion criteria, 119 patients were included in this study which as the infection group, including 99 males and 20 females, with an average age of 59.08 ± 12.30 years. According to the same sex, same department, same age (±2), same operation time (±2w), 1 : 1 matched uninfected patients, there were another 119 cases which served as control group. This study was approved by the Medical Ethics Committee.

2.1.1. Inclusion Criteria. (1) Interventional surgery performed in this hospitalization; (2) According to the diagnostic or clinical criteria, BSI was confirmed by clinical symptoms, signs and pathogenic bacteria examination; (3) Complete case data.

2.1.2. Exclusion Criteria. (1) No operation in this hospitalization; (2) BSI before operation; (3) Patients had multiple hospitalizations and repeated BSI during the study; (4) Combine community infection; (5) Blood culture was identified as contamination by diagnostic criteria.

2.1.3. Diagnostic Criteria for BSI. According to CDC diagnostic criteria for BSI in the United States [9]: Laboratory BSI diagnosis should meet the criteria as follows: (1) Blood culture is positive once or more, and the positive pathogen has nothing to do with other infected sites or can be sure coming from other infected sites because of the same bacteria detected. (2) The patient has at least one of the following symptoms: fever (>38°C), shivering or hypotension. At least one of the following items can be satisfied: (1) If the blood culture is a common skin microorganism, the blood culture should be positive twice or more at different times. (2) If the blood culture is a common skin bacteria and the blood culture is positive only once, the same pathogen which is positive by venous catheter culture is needed. (3) Blood antigen test was positive (e.g., *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Neisseria meningitidis*, or Group B streptococcus), and the symptoms, signs and laboratory results could not be explained by infection in other parts.

2.1.4. Blood Culture Contamination Criteria. It should meet at least one of the following criteria [10, 11]: (1) No obvious fever and risk factors (such as low immune function or invasive operation); (2) Despite the above risk factors, many subsequent blood cultures proved to be other pathogens; (3) Ineffective empiric antibiotic therapy; (4) Fever can be explained by other reasons such as immunity of tumors, and no obvious signs of infections. (5) Laboratory standards, such as extended incubation of blood culture positive; continuous multiday culture only once for bacteria; blood culture once to isolate more than two kinds of skin flora.

Table 1: Occurrence of BSI in interventional patients in different years.

Year	No. of patients	No. of HAIs	No. of BSI	Proportion %	Rate %
2013	3911	51	24	47.06	0.61
2014	4013	51	19	37.25	0.47
2015	4155	48	22	45.83	0.53
2016	4160	54	28	51.85	0.67
2017	4577	63	40	63.49	0.87
2018	4585	81	41	50.62	0.89
Total	25401	348	174	50.00	0.69

2.2. Methods. Through the "real-time monitoring system of healthcare-associated infections" to collect the case information of patients with BSI in interventional department during the research period, design questionnaires, using them after evaluation by experts, checking the cases one by one in detail, and register the patients' gender, age, basic diseases, disease diagnosis, time of admission and exit, type of operation, past history, and occurrence of BSI (date, pathogenic bacteria, etc.), time and type of antibiotic prophylaxis use, etc. Total 18 relevant factors were counted.

2.3 Statistical Analysis. Differences in categorical variables were assessed using Pearson χ^2 test or Fisher's exact test (when expected cell frequencies were <5). Paired nominal-scale data were used *t*-Test. Multivariate Analysis were used multi-variate logistic analysis. SPSS version 19.0 was used for all statistical analyses. A two-tailed P value of <0.05 was considered to be statistically significant.

3. Results

3.1. Incidence of BSI. Total 25401 patients were monitored in 6 years, and 348 cases (including lower respiratory infection 51 cases, surgical site infection 34 cases, urinary tract infection 25 cases, BSI 174 cases, upper respiratory infection 19 cases, alimentary infection 17 cases, etc.) were infected. Among them, 174 cases (accounted for 50.0%) had BSI, and the overall incidence of BSI was 0.69%. From 2013 to 2018, with the increase of the number of hospitalized patients, the incidence of BSI also increased significantly, but with no statistic difference ($\chi^2 = 9.770$, $P > 0.05$) (Table 1).

3.2. Pathogenic Bacteria. 157 pathogenic bacteria were detected from 119 patients. The majority (88, 56.05%) were Gram-positive bacteria. The most prevalent isolates were Coagulase-negative *Staphylococcus* (CNS) (48, 30.57%) and *Escherichia coli* (32, 20.38%). Total 116 drug-resistant bacteria were detected from 157 (73.88%) (Table 2).

3.3. Description of Clinical Characteristics. Analysis of the clinical data of the infected cases, showed that the majority of the patients were males, accounting for 83.19%. Most of them were over 60 years old (55.46%). 38.66% of them had combined

Table 2: Detection of pathogenic bacteria in blood culture.

Pathogenic bacteria	No. (%) of bacteria	No. (%) of drug-resistant bacteria
Gram-positive bacteria	88/157 (56.05%)	64/88 (72.73%)
Coagulase-negative *Staphylococcus*	48/157 (30.57%)	39/48 (81.25%)
Micrococcus luteus	9/157 (5.73%)	7/9 (77.78%)
Enterococcus faecium	14/157 (8.92%)	7/14 (50.0%)
Gram-positive bacilli	9/157 (5.73%)	9/9 (100.0%)
Enterococcus faecalis	4/157 (2.55%)	1/4 (25.0%)
Staphylococcus aureus	2/157 (1.27%)	0
Others	2/157 (1.27%)	1/2 (50.0%)
Gram-negative bacteria	68/157 (43.31%)	32/68 (47.06%)
Escherichia coli	32/157 (20.38%)	14/32 (43.75%)
Klebsiella pneumoniae	10/157 (6.37%)	6/10 (60.0%)
Enterobacter cloacae	8/157 (5.10%)	3/8 (37.5%)
Pseudomonas aeruginosa	6/157 (3.82%)	1/6 (16.67%)
Acinetobacter baumannii	3/157 (1.91%)	3/3 (100.0%)
Others	9/157 (5.73%)	5/9 (55.56%)
Fungus	1/157 (0.64%)	0
Candida glabrata	1/157 (0.64%)	0

Note: definition of drug-resistant of major bacteria, (1) Oxacillin-resistant coagulase-negative Staphylococci, (2) methicillin-resistant *Staphylococcus aureus*, (3) carbapenem resistant *Escherichia coli*, (4) carbapenem resistant *Klebsiella pneumonia.*

basic diseases. 41.18% of the patients had complications with acute or chronic biliary diseases before operation. 43.70% of the patients had undergone surgical resection of primary tumors. The average incidence of BSI was 2.5 ± 2.306 times, the first time intervention was the most common, accounting for 47.90%. The average incidence of BSI was 3.02 ± 2.393 days, accounting for 53.78%. Primary BSI accounted for 74.79%, secondary BSI accounted for 10.08%, and transient bacteremia accounted for 15.13%. HCC was assessed as the main risk factor associated with infection (87.39%). Transarterial chemoembolization (TACE) was the main risk associated procedure (46.22%).

Among them 98.32% of the patients had used antibiotics before the operation. The total prophylactic antibiotic usedays were 3.01 ± 2.444. The highest used antibiotic grade was restricted antibiotics (55.46%), Cephalosporin was the most commonly used antibiotics classification (70.08%), the second was Quinolones (5.13%). 23.08% of the patients used antibiotics jointly, the major combined was Cephalosporin and Nitroimidazoles. 13.68% of the patients had changes in type of antibiotic treatment during the prophylactic use (Table 3).

3.4. Relevant Risk Factors Analysis. Sex, age, weight, multiple clinical diseases, primary diseases, surgical procedures, surgical staff, frequency of chemotherapy, frequency of interventional operations, biliary complications, had undergone surgery, time of prophylactic antibiotic use, types. etc. 18 indicators were included in χ^2 test or t-test. The results showed that 6 indicators were $P < 0.05$, respectively: disease diagnosis, had undergone surgery, biliary complications, prophylactic antibiotic use, replacement of antibiotics, number of interventional operations and days of prophylactic antibiotic use (Table 4).

3.5. Multivariate Analysis. Six meaningful indicators of single factor analysis were included in logistic analysis. Variables were screened step by step by the forward method. The regression equation was fitted with the retention condition $P < 0.05$. The factors that finally entered the fitting model were: days of prophylactic antibiotic use, and replacement of antibiotics (Table 5).

4. Discussion

With the rapid development of interventional surgery in the medical field, it is used more and more widely in clinic. With the increasing numbers of operations, it is particularly important to effectively control HAIs and improve medical quality [1, 2]. China has a high incidence area of hepatocellular carcinoma. At present, the incidence and mortality rate of hepatocellular carcinoma accounts for 50.0% worldwide. Surgical resection and recurrence are the most widely used and effective options. As a minimally invasive technique, interventional chemotherapy for HCC directly acts on the lesion by introducing corresponding chemotherapeutic drugs and biological agents through catheters. The goal is more accurate, and local effective concentration is higher, and also it is safer and more effective than systemic chemotherapy [12, 13]. Although it is recognized as a safe treatment, various complications such as self-limiting postembolization syndrome, biloma, BSI, hepatic failure, cholecystitis, gastrointestinal bleeding, pancreatitis, renal failure, liver infarction, and liver abscesses have been reported [14–16]. According to the data of our study, the overall incidence of BSI was 0.69%, accounting for 50% of the total infection sites, most of the BSI patients were HCC patients receiving TACE, accounting for 46.22%. BSI had become the major type of infection after interventional surgery in a hospital. The occurrence of BSI has aggravated the medical burden and seriously affected the prognosis of the disease. This needs further attention and study.

Table 3: Clinical characteristics of patients with BSI after interventional surgery.

Variables	Grouping, No (%). of cases	
Gender	Male, 99 (83.19%)	Female, 20 (16.81%)
Age	≤40, 10 (8.4%)	41~60,43 (36.13%), ≥60,66 (55.46%)
Combined basic diseases	Yes, 46 (38.66%)	No, 73 (61.34%)
Biliary complications	Yes, 49 (41.18%)	No, 70 (58.82%)
Had undergone surgery	Yes, 52 (43.70%)	No, 67 (56.30%)
No. of interventional operations	once, 57 (47.90%)	No. 2~5, 52 (43.70%), No. >5, 10 (8.40%)
The first positive blood culture day after operation	≤2 days, 64 (53.78%)	3–5 days, 42 (35.29%), >5 days, 13 (10.92%)
BSI property	Primary BSI, 89 (74.79%)	Secondary BSI, 12 (10.08%), Transient bacteremia, 18 (15.13%)
Disease diagnosis	HCC, 104 (87.39%)	Others, 15 (12.61%)
Term of operation	Microwave ablation, 38 (31.93)	TACE, 55 (46.22%), RFCA, 14 (11.76%), Others, 12 (10.08%)
Prophylactic antibiotic use	Yes, 116 (98.32%)	No, 3 (1.68%)
Use of antibiotics on the day of operation	Yes, 103 (86.55%)	No, 16 (13.45%)
Antibiotic grade	Unrestricted, 4 (36.79%)	Restricted, 66 (55.46%), Special, 7 (5.88%)
Days of prophylactic antibiotic use	1 day, 44 (36.79)	2~5 days, 61 (51.26%), >5 days, 14 (11.76%)
Combined prophylactic antibiotic use	Yes, 27 (23.08%)	No, 90 (76.92%)
Replacement of antibiotics	Yes, 16 (13.68%)	No, 102 (87.18%)

Note: RFCA = Radiofrequency Ablation; (1) Primary BSI as blood culture is positive once or more, and the positive pathogen has nothing to do with other infected sites; (2) Secondary BSI as blood culture is positive once or more, and the positive pathogen can confirm come from other infected sites. Because they have detected the same bacteria; (3) Transient bacteremia as blood culture is positive once or more but without any symptoms.

Table 4: Relevant factors of BSI in patients undergoing interventional surgery.

Variables	Experimental group ($N = 119$)	Control group ($N = 119$)	χ^2	P
Disease diagnosis				
HCC	104	81	15.801	0.001
Others	15	38		
Had undergone surgery				
Yes	53	29	10.717	0.001
No	66	90		
Biliary complications				
Yes	49	23	13.461	0.001
No	70	96		
Prophylactic antibiotic use				
Yes	117	96	19.710	0.001
No	2	23		
Replacement of antibiotics				
Yes	16	4	5.697	0.019
No	100	92		
No. of interventional operations	2.5 ± 3.880	1.96 ± 1.729	5.833	0.04
Days of prophylactic antibiotic use	3.01 ± 2.444	4.73 ± 2.469	0.031	0.01

A total of 157 strains of bacteria were detected in blood culture, of which 88 were Gram-positive bacteria (56.05%), 68 were Gram-negative bacteria (43.31%) and 1 was fungus (0.64%). It was reported that Gram-negative bacilli had the highest detection rate of BSI [17]. However, in recent years, with the wide application of broad-spectrum antibiotics and the increase of indwelling catheters and invasive operations, the pathogenic structure of BSI has changed greatly, and the detection rate of Gram-positive cocci has gradually increased [18]. Relevant studies in the United States and Europe had reported that more than 65.0% of BSI was caused by Gram-positive bacteria. Coagulase-negative *Staphylococcus* (CNS), *Staphylococcus aureus* and *Enterococcus* were the most common pathogens in BSI [19]. CNS is a weak virulent bacterium represented by *Staphylococcus epidermidis* and *Staphylococcus hemolyticus*. Before 1970s, CNS was often regarded as a contaminant bacterium in clinic [20]. However, in recent years, monitoring CNS has accounted for 1–3rd of BSI in hospital [21]. 72 cases CNS were detected in this study. On the basis of strict application of the diagnostic criteria, 24 cases of CNS with suspected contamination were eliminated. The contamination rate of CNS was 33.3%. The hospital strictly required multiple samples of blood to reduce the contamination rate as much as possible, but there were still instances of blood culture contaminations. Infection control preventive measures including appropriate choice of disinfection products, cleaning and disinfection surgical procedure protocols and blood culture collection protocols must be implemented consistently in accordance to current regulatory guidelines. A total of 48 CNS (54.55%) causing BSI were obtained after eliminating contamination. Among them, 39 oxacillin-resistant coagulase-negative staphylococci were detected, the detection rate was 81.25%, slightly higher than the rate above 70% be reported by Ledha A [22]. It is suggested that CNS is the most important bacteria in BSI, and the drug resistance is emerging as serious complication of antibiotic therapy. Although the United States

Table 5: Multivariate logistic of BSI in patients undergoing interventional surgery.

Variables	*B*	S.E	Wals	*P*	OR	95% CI
Days of prophylactic antibiotic use	.461	.090	26.240	.001	1.586	1.329~1.892
Replacement of antibiotics	2.591	.925	7.846	.005	13.349	2.177~81.835

reported [23] that *Staphylococcus aureus* accounted for 20.0% of BSI in hospitals were the most important Gram-positive pathogenic bacteria, but only two strains of *Staphylococcus aureus* were isolated in our study, and none of them were resistant to antibiotics. It was worth noting that 9 strains of Micrococcus (7 strains of drug-resistant) and 9 strains of drug-resistant Gram-positive bacilli were also detected in this group, and the infection caused by them was transient asymptomatic bacteremia.

Escherichia coli had been detected 32 strains (47.06%) which was the dominant strain of gram-negative bacilli. It was reported that the clinical detection rate of *Escherichia coli* in BSI is 18.7% [23]. The production of Extended-Spectrum Beta-Lactamases (*ESBLs*) was the main resistance mechanism of *Escherichia coli*, and its detection rate continues its increasing incremental rise. The positive rate of *ESBLs* was 27.8%–30.1% [19, 23]. In our study 14 of the 32 strains of ESBLs accounted for 43.75%. 7 positive were for ESBLs in another 10 strains of *Klebsiella pneumoniae*, and five strains (3 strains were resistant) were detected in 2018, which was consistent with the increase of detection rate of Carbapenem-resistant *Klebsiella pneumoniae* in recent two years [24]. Increasing concern about virulence of KPC requires greater attention and further study.

Male patients were mainly infected in this group of data, which was 3 times compared with females, with no statistical significance; the patients who were older than 60-years-old and with HCC were the majority (55.46%). The infection rates of HCC and other diseases were significantly different ($P < 0.05$), which was related to the infection of the biliary tract system caused by liver adjacent to the biliary tract system, puncture and drainage, or the patients complicated with biliary tract slowness. Bacteremia risk factors associated with had biliary complications (41.18% in this study, $P < 0.05$), puncture of blood vessels to break the infected bile into the blood system [25]. 46.22% of the infected patients received perfusion and chemoembolization, and the necrotic liver tissue after embolization or chemoembolization was other major risks associated with infection, while the blood supply artery of liver tumors was minimized during the operation. At the same time, it will lead to insufficient blood supply to the intrahepatic bile duct, which will eventually lead to bile duct injury and the infection of biliary pathogens in the liver tissue [26]. Gram-negative bacteria are thought to be mostly associated with infections through this channel. In addition, whether or not had undergone surgery was performed the related factor of BSI ($\chi^2 = 10.717$, $P < 0.05$). This study also concluded that the number of interventional operations was related to the incidence of infection ($P < 0.05$). The average number of interventional operations occurred in patients with 2.5 ± 2.306 interventions, with the first interventional operation accounting for 47.90%. Whether this is related to the body's initial stress response needs further study. Among 119 cases of BSI, 89 cases were primary BSI, accounting for 74.79%, which occurred within 48 h after interventional operations, accompanied by fever, chills and inflammatory factors elevated. 12 cases were secondary BSI, accounting for 10.08%, 11 cases were second to surgical site infection, 1 case was second to lower respiratory infection. 18 cases were transient bacteremia, accounting for 15.13%, which had only a transient fever and recovered without the replacement of antibiotic for therapy.

Because of the high rates of HAIs in interventional surgery, antibiotics should be used routinely, but some studies have shown that prophylactic antibiotic before interventional surgery can not prevent the occurrence of infection [27]. Pathogenic bacteria can still be cultured from the blood, there are several possibilities: inadequate dosage of antimicrobial agents; irregular medication time; potential for lapse or break in aseptic technique during surgical procedures [28]. In these data, 98.32% of the patients had used antibiotics before operation, 88.89% were given antibiotics for the first time in 0.5–2 hours before operation. The highest used antibiotic grade was restricted antibiotics (55.46%), Cephalosporins was the most commonly used antibiotics classification (70.08%), Cefmetazole and Cefuroxime were the main drugs, but there was no statistical difference in both use level and class of antibiotics ($P > 0.05$). 23.08% of the patients used antibiotics jointly and 13.68% of them changed antibiotics during preventive use. The total prophylactic antibiotic days were 3.01 ± 2.444, 67.22% of the patients were treated with a higher level of antibiotics immediately after infection. Compared with the control group, the overall incidence of BSI in the replacement of antibiotics was higher ($P < 0.05$), and the overall time of prophylactic antibiotic use in the infected group was shorter than that in the control group ($P < 0.05$). These two points were the independent risks of infection. We suspect that inadequate prophylactic antibiotic or replacement of antibiotics may be the main cause of BSI after interventional surgery. Therefore, this study suggests the associated risk factors of hepatocellular carcinoma biliary complications, had undergone surgery, etc., the timing of antibiotic prophylaxis including proper length of antibiotic administration and appropriate antibiotic selection to single antibiotic class to avoid unnecessary antibiotic therapy with multiple changes and replacement in therapy prior to and after surgical procedure.

However suggested changes in prophylactic antibiotic therapy cannot replace standard infection control measures of cleaning and disinfection and sterilization of equipment and instrumentation. Preventive actions to strengthen the perioperative environment, defined cleaning and disinfection protocols, post-operative protocols and other infection control and antibiotic stewardship based measures can reduce the incidence of HAIs after surgical procedures.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Yanling Bai and Zhigang Zheng contributed equally to this work.

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Research Article

Evaluation of Drug Susceptibility of Microorganisms in Odontogenic Inflammations and Dental Surgery Procedures Performed on an Outpatient Basis

Mateusz Bogacz,[1] **Tadeusz Morawiec**,[1] **Joanna Śmieszek-Wilczewska**,[1] **Katarzyna Janowska-Bogacz**,[2] **Anna Bubiłek-Bogacz**,[1] **Rafał Rój**,[3] **Karolina Pinocy**,[1] **and Anna Mertas**[4]

[1]*Department of Oral Surgery, School of Medicine with the Division of Dentistry in Zabrze, Medical University of Silesia in Katowice, Pl. Akademicki 17, Bytom 41-902, Poland*
[2]*Department of Conservative Dentistry with Endodontics, School of Medicine with the Division of Dentistry in Zabrze, Medical University of Silesia in Katowice, Plac Akademicki 17, Bytom 41-902, Poland*
[3]*Department of Prosthetic Dentistry, School of Medicine with the Division of Dentistry in Zabrze, Medical University of Silesia in Katowice, Plac Akademicki 17, Bytom 41-902, Poland*
[4]*Department of Microbiology and Immunology, School of Medicine with the Division of Dentistry in Zabrze, Medical University of Silesia in Katowice, Jordana 19, Zabrze 41-808, Poland*

Correspondence should be addressed to Mateusz Bogacz; mateusz.bogacz@gmail.com

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Bacterial infections are the most common cause of purulent soft tissue inflammations in the head and neck area. These bacteria are also responsible for the majority of inflammatory complications after third molar removal. The key to success of antibacterial treatment in both cases is the use of an appropriate antibacterial agent. The aim of the study was to evaluate the susceptibility profile of bacteria isolated from material collected from patients with intraoral odontogenic abscesses. The test material consisted of swabs taken from the odontogenic abscesses, after their incision and drainage. Another swab was collected from the lesion area, 10 days after the initial visit. Results were compared with an identical study conducted on a control group of healthy patients, who had undergone third molar removal. Bacteria identified in this study consisted of aerobic and anaerobic strains, both Gram-positive and Gram-negative. According to the EUCAST guidelines, none of the tested antibiotics was recommended for all identified bacteria. The percentage of bacterial strains sensitive to amoxicillin and clavulanic acid was 78.13% and 81.48% in the study and control groups, respectively, whereas, the percentage of those sensitive to clindamycin was 96.43% and 80.00%, respectively. For Gram-negative aerobic bacteria, gentamicin and ciprofloxacin were among medications affecting all cultured species. 100.00% of strains were found to be susceptible to these antibiotics. Statistically significant relationship between the presence of Gram-negative aerobic strains and the occurrence of complications was found. In the case of the most frequently occurring bacteria in the study, amoxicillin with clavulanic acid and clindamycin were shown to be very effective. In cases of severe purulent odontogenic inflammations, it is recommended to use a combination of antibiotics. Amoxicillin with ciprofloxacin and clindamycin with cefuroxime seem to be the proper choices based on the results of this study.

1. Introduction

Bacterial infections are the most common cause of purulent soft tissue inflammations in the head and neck area [1]. Their occurrence is favoured by a large variety of oral microbiota and lesions of dental tissues and the periodontium [2, 3]. The causes of infections are divided into odontogenic and nonodontogenic, with 70–90% of cases belonging to the first

group. The most common odontogenic causes are gangrenous teeth, complicated third molar eruption, infected dental cysts, residual tooth roots, and complications after endodontic treatment [4–6]. The most frequent cause of the development of periapical inflammatory changes is pulpitis, which results from negligence in conservative treatment [7, 8]. The bacterial antigens present in the inflamed pulp tissue stimulate the specific and nonspecific immune responses of the body, but it is usually not possible to completely eradicate the infection [9]. A chronic inflammatory change develops in the periapical region of the infected tooth. The central part of the lesion exhibits the largest accumulation of neutrophilic granulocytes, forming foci of colliquative necrosis, in which purulent exudate accumulates. Distribution of periodontal collagen fibres causes merging of smaller purulent foci, which ultimately leads to the formation of a periapical abscess [10]. Chronic inflammations are usually asymptomatic and almost always lead to bone resorption around the tooth root, giving characteristic lucencies in the X-ray image. This is not the case with acute inflammations, the course of which is most often associated with severe pain and swelling of soft tissues. Acute inflammations do not show any characteristic features in the X-ray image. In some cases, they can manifest themselves in the widening of the periodontal ligament space [11]. However, the probable causes of such inflammations are often visible—most often deep carious lesions, extensive fillings, including the pulp chamber or in the close vicinity of it. Acute inflammation can be both a primary condition and exacerbation of chronic inflammation. It is characterized by a fast course, during which there is no natural barrier to the spread of infection. This type of inflammation is considered more dangerous, as microorganisms penetrating the periapical tissue can spread to other parts of the head and neck [9, 11, 12]. Clinically, we distinguish different forms of odontogenic inflammation, which, depending on the severity of the disease, differ significantly in terms of symptoms reported by the patient and their treatment. Root canal treatment is recommended for the management of periapical inflammations and abscesses. In the case of purulent soft tissue inflammation, it is necessary to perform intra- or extraoral incisions to obtain effective drainage. Seton placed inside the abscess is replaced daily until the complete evacuation of the purulent exudate, and the patient attends checkups until the clinical condition is significantly improved [13–15]. In the case of oral inflammation with symptoms that might be life-threatening, the treatment should be complemented by empiric antibiotic therapy. The microbiome of the oral cavity consists of aerobic and anaerobic species, both Gram-positive and Gram-negative; therefore, no antibiotic is effective against all of them. The key to success in empirical antibiotic therapy is to identify those bacteria species that are most frequently related to ongoing infection and assess their susceptibility to drugs that can be used efficiently in everyday dental practice. Antibiotics are often used in dentistry before planned surgery in order to minimize the risk of postoperative infections. This procedure is frequently employed in the impacted third molar (ITM) surgery. Compared to simple tooth extraction, the ITM surgery has a greater risk of penetration of microorganisms present in the oral cavity into the tissues. Such a condition can impair wound healing; in some situations, it can also lead to the development of a generalized infection. This is particularly dangerous for patients with systemic diseases and hence an already weakened immune system. In order to ensure proper healing, patients need to maintain proper oral hygiene (including removal of dental plaque) prior to the treatment. It is also necessary to instruct the patient on the principles of oral hygiene. The patient compliance with the recommendations should be checked according to appropriate indicators, including the Approximal Plaque Index (API). Patients who are eligible for elective ITM surgery should not have clinical symptoms of an ongoing inflammatory process. Otherwise, it is necessary to precede the surgical procedure with an appropriate conservative treatment.

2. Materials and Methods

2.1. Study and Control Groups and Patient Examination. The study included 52 patients, who were divided into two groups:

(1) Study group (26 patients)—patients who were diagnosed with the following:
   (a) Submucous abscess, requiring removal of causative teeth, as well as soft tissue incision and setoning—2 women and 13 men
   (b) Periapical abscess, requiring only removal of causative teeth (purulent exudate drained through the alveolus)—3 women and 8 men

(2) Control group (26 patients, including 19 women and 7 men)—patients without acute inflammation, referred for a planned ITM surgery

A dental diagram was made during the study, based on which the DMF (Decay-Missing-Filled) Index was calculated by counting the number of decayed (*D*), missing (*M*) due to caries, and restored (filled—*F*) teeth in each patient. Treatment Index was also calculated for each patient (Figure 1).

The Approximal Plaque Index (API) was also calculated at each visit. Before the procedure, patients in both groups underwent a radiological examination in the form of point, panoramic, and, if necessary, volumetric tomography. The examinations included the general state of health of the patients, medications they were taking, and the current treatment of the existing inflammation. The first stage of treatment in patients from the study group diagnosed with submucous, subperiosteal, or subcutaneous abscess involved incision of the purulent lesion to evacuate its content. Due to contraindications to endodontic treatment (poor oral hygiene and extensive tooth crown damage), the next stage involved removal of the causative tooth. In the case of periapical abscesses, the surgical part of the treatment consisted only of removing the causative tooth. The next stage of the

$$\text{Treatment index} = \frac{F}{D+F} = \frac{\text{Teeth restored}}{\text{Teeth restored} + \text{Teeth decayed}}$$

Figure 1: Calculating Treatment Index based on the *F* and *D* scores from the DMF Index.

procedure was collection of swabs for microbiological examination. Material collected for testing was purulent exudate from inflammatory foci in oral tissues. Directly prior to the collection, the lesion area was isolated with sterile gauze and disinfected. The first portion of purulent content was removed, and then a smear swab was taken from the deepest possible site (using the sterile swab) (study I). In cases requiring extraction of the causative tooth with no indications for incision, the place of swab collection was the deepest possible spot of tooth alveolus, after removal of the first portion of purulent exudate. The swabs were placed in a transport medium for aerobic and anaerobic microorganisms. In accordance with routine procedures, patients were required to report for control visits every day over the next several days to assess the healing of the lesion. At each visit, the general and local condition of the patient was assessed and the seton was changed. Healing of these types of lesions usually takes 7 to 10 days. The last visit was set for 10 days after the beginning of the treatment, and a control swab from the area of the lesion was collected (study II).

The control group consisted of patients with a planned ITM surgery. On the day of the surgery, a clinical and radiological examination was carried out (as described above), followed by collection of a swab for microbiological examination from the surgical area (study I). The next step was to perform the ITM surgery, and the procedure was carried out as follows:

(1) Incision and detachment of the mucoperiosteal flap
(2) Exposition of the impacted tooth and its separation with a burr
(3) Removal of the tooth along with the surrounding pathological lesions (follicular cysts, tooth follicle and granulomatous lesions)
(4) Wound management with sutures and pressure dressing

The control visit was set for the 10^{th} day after the procedure. After evaluating the healing of the wound, a swab was taken for microbiological tests from the surgical area (study II), and then sutures were removed under topical anaesthesia.

2.2. Antibiotic Therapy—Indications. In the study group, every patient diagnosed with oral inflammation accompanied by possibly life-threatening symptoms (rapidly growing face swelling, trismus, significant enlargement and painfulness of the surrounding lymph nodes, impaired swallowing, and breathing), systemic symptoms such as tachycardia (with pulse over 100 bpm), or increased body temperature underwent treatment complemented by an empiric antibiotic therapy.

In the control group, every patient in whose case the ITM surgery was associated with disruption of the bone tissue continuity and every patient for whom the surgery took more than 30 minutes also underwent treatment complemented by an empiric antibiotic therapy.

In the case of indications for the implementation of antibiotic therapy, one of the following two antibiotics was used:

(1) Amoxicillin (875 mg) with clavulanic acid (125 mg), 1 tablet every 12 hours for 6 days
(2) Clindamycin (600 mg), 1 tablet every 12 hours for 6 days

In the present study, indications for the initiation of antibiotic therapy were found in 19 out of 26 patients (73.08%) in the study group and in 21 out of 26 (80.77%) patients in the control group.

2.3. Microorganism Identification and Evaluation of Drug Susceptibility. Material collected from the patients was delivered to the Microbiological Laboratory of the Chair and Department of Microbiology and Immunology in Zabrze, Medical University of Silesia in Katowice, where microbiological tests were carried out. The time from material collection until delivery to the laboratory did not exceed 2 hours. Microbiological tests were carried out using classic methods used in microbiological diagnostics. The material was seeded on appropriate culture media to amplify and isolate pure microbial cultures. Aerobic bacteria were grown on solid Columbia agar with 5% sheep blood at 37°C. Anaerobic bacteria were grown on a solid Schaedler K3 with 5% sheep blood at 37°C under anaerobic conditions obtained with the use of GENbag anaer kits (Biomerieux, Marcy-l'Etoile, France). After isolation and multiplication of cultivated microbial strains, species identification was performed using the following reagent kits (Erba-Lachema, Brno, Czech Republic): ENTEROtest 24 N, NEFERMtest 24 N, STREPTOtest 24, STAPHYtest 24, ANAEROtest 23, OXItest, PYRAtest, as well as Erba-Lachema's TNW Lite 6.5 software (Brno, Czech Republic). The following biochemical tests were also used (Biomerieux, Marcy-l'Etoile, France): Katalaza and Slidex Staph Kit. The performance, reading, and interpretation of test results were carried out in accordance with the recommendations of manufacturers of diagnostic reagent kits.

Bacterial drug susceptibility was determined using the Kirby–Bauer disk diffusion method [16] and Etest method. The implementation of this stage of the study and the interpretation of the obtained results were in accordance with the current EUCAST (European Committee on Antibiotic Susceptibility Testing) recommendations [17]. Twelve antibiotics belonging to following different classes were used in the form of discs (Oxoid Limited, Basingstoke, UK) and/or Etests (Biomerieux, Marcy-l'Etoile, France): (a) penicillins: benzylpenicillin 1 unit (P), amoxicillin with clavulanic acid 20–10 μg (AUG), piperacillin with tazobactam 30–6 μg (TZP), and ampicillin 10 μg for Enterobacterales or 2 μg for the other bacterial species (AM); (b) cephalosporins:

cefuroxime 30 μg, the 2nd generation (CXM), and cefepime 30 μg, the 4th generation (FEP); (c) fluoroquinolone: ciprofloxacin 5 μg (CIP); (d) aminoglycoside: gentamicin 10 μg (G); (e) glycopeptide: vancomycin 5 μg (Va); (f) lincosamide: clindamycin 2 μg (CC); (g) nitroimidazole: metronidazole—only Etest (MZ); and (h) aminopyrimidine with sulphonamide: trimethoprim-sulfamethoxazole 1,25–23,75 μg (biseptol—Bs).

Statistical analyses were carried out using the Statistica PL v. 13 software (Statsoft, Kraków, Poland), assuming the level of significance at $\alpha = 0.05$.

3. Ethical Approval

All subjects gave their informed consent for inclusion before they participated in the study. The study protocol was approved by the Ethics Committee of Śląska Izba Lekarska in Katowice (project identification code: 45/2015).

4. Results and Discussion

4.1. Characteristics of the Studied Population. The study group consisted of 26 patients, including 5 women and 21 men, aged 21 to 82 years (47.46 ± 14.49). The control group consisted of 26 individuals, including 19 women and 7 men, aged 13 to 82 years (33.04 ± 16.75). The average age of patients in the study group was higher than in the control group, and the difference was statistically significant ($p = 0.0017$). Data on the age of patients are shown in Table 1.

In the present study, men constituted a majority in the study group (80.77%). The relationship between sex and the occurrence of purulent odontogenic inflammations in the soft tissues of the head and neck area is highly statistically significant ($p = 0.0001$). The results are presented in Table 2.

The DMF Index was 18.96 ± 4.94 for patients in the study group and 13.96 ± 5.52 for those in the control group. Lower values of the DMF Index were found in patients in the control group. The differences were statistically significant ($p = 0.0012$). Treatment Index values for patients in the study and control groups were 0.50 ± 0.32 and 0.67 ± 0.26, respectively. Higher values of the Treatment Index were found in patients in the control group. The differences were statistically significant ($p = 0.0375$). The results are shown in Figure 2.

The API values in study I were 66.50 ± 22.08 for patients in the study group and 38.58 ± 26.11 for patients in the control group, whereas in study II they were 68.88 ± 22.28 and 47.85 ± 24.82, respectively. The API value was lower in the control group than in the study group. The difference was statistically significant, both in study I ($p = 0.0001$) and in study II ($p = 0.0023$). There was also a statistically significant increase in the API in the control group between study I and study II ($p = 0.0043$). The data are presented in Figure 3.

4.2. Drug Susceptibility Assessment. A total of 67 strains from 31 species of potentially pathogenic microorganisms from the material collected from the tested subjects were assessed.

Table 1: Age of patients in the study and control groups.

	Study group (mean ± SD)	Control group (mean ± SD)	p
Age of patients (years)	47.5 ± 14.5	33.0 ± 16.8	0.0017

Table 2: Sex of patients in the study and control groups.

	Study group	Control group	Total
Women	5	19	24
Percentage (%)	19.23	73.08	46.15
Men	21	7	28
Percentage (%)	80.77	26.92	53.85
Total	26	26	52

The drugs studied included: penicillin (P), amoxicillin with clavulanic acid (AUG), vancomycin (Va), piperacillin with tazobactam (TZP), clindamycin (CC), metronidazole (MZ), gentamicin (G), biseptol (Bs), cefuroxime (CXM), ciprofloxacin (CIP), cefepime (FEP), and ampicillin (AM). Patients participating in the study were given amoxicillin with clavulanic acid (22 patients) or clindamycin (19 patients). No indications for antibiotic therapy were found in 11 patients. When assessing the sensitivity of bacteria according to the EUCAST guidelines, 100% sensitivity to the tested antibiotics was found in the case of the following drugs:

(i) In the study group: gentamicin, cefuroxime, and ciprofloxacin

(ii) In the control group: gentamicin and sulfamethoxazole with trimethoprim (biseptol) and cefepime

Comparing the sensitivity to drugs used in patients in this study, the percentage of bacterial strains sensitive to amoxicillin and clavulanic acid in the study and control groups was 78.13% and 81.48% ($p = 0.75$), respectively, and the percentage of bacterial strains sensitive to clindamycin was 96.43% and 80.00% ($p = 0.17$). The studied bacteria were found to be least sensitive to ampicillin, resulting in a total lack of sensitivity in the case of the study group and 14.29% of sensitive strains in the case of the control group. Among Gram-positive anaerobic bacteria, the highest percentage of susceptible strains was found in the case of amoxicillin with clavulanic acid and metronidazole (100.00%), and clindamycin was found to be effective in 78.57% of strains. In the case of Gram-negative anaerobic bacteria, the highest percentage of susceptible strains was also found in the case of amoxicillin with clavulanic acid, followed by clindamycin (98.32% of susceptible strains). According to the EUCAST 8.1 guidelines, in the case of infections caused by anaerobic bacteria, gentamicin, biseptol, cefuroxime, ciprofloxacin, cefepime, and ampicillin are not recommended. In the case of Gram-positive aerobic bacteria, the only drugs recommended for all cultured strains are clindamycin and sulfamethoxazole with trimethoprim. 88.89% of strains were found to be susceptible to clindamycin and 66.67% to sulfamethoxazole with

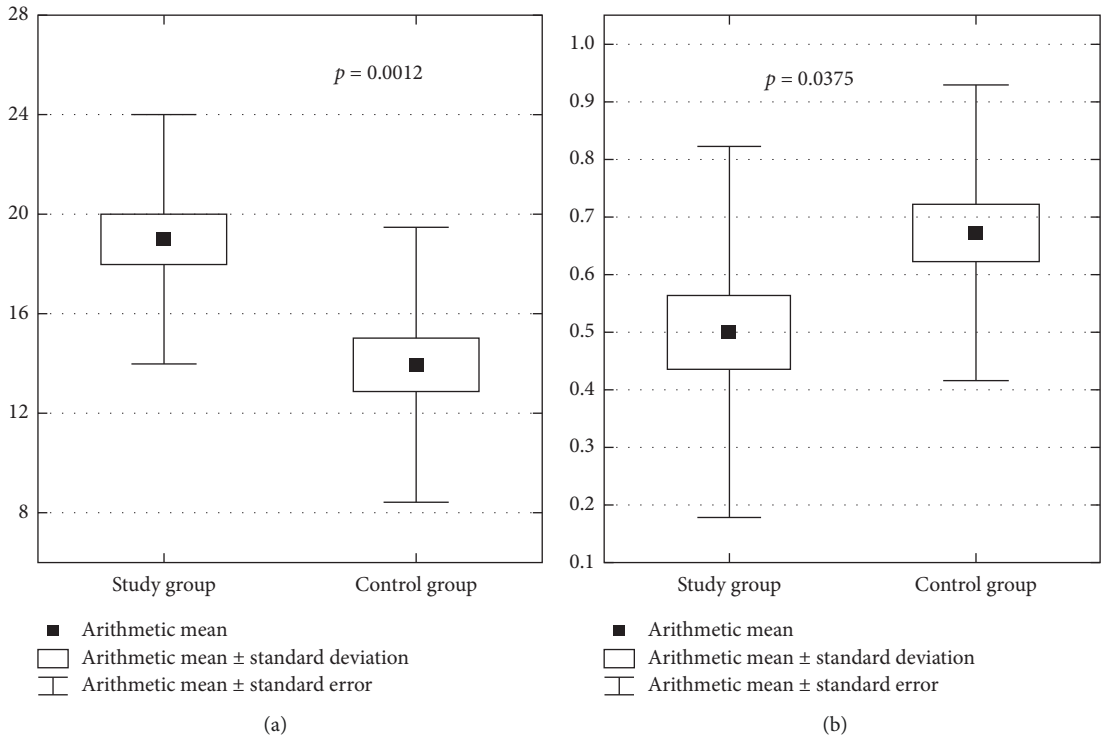

Figure 2: (a) DMF Index values in both groups of patients and (b) Treatment Index values in both groups of patients.

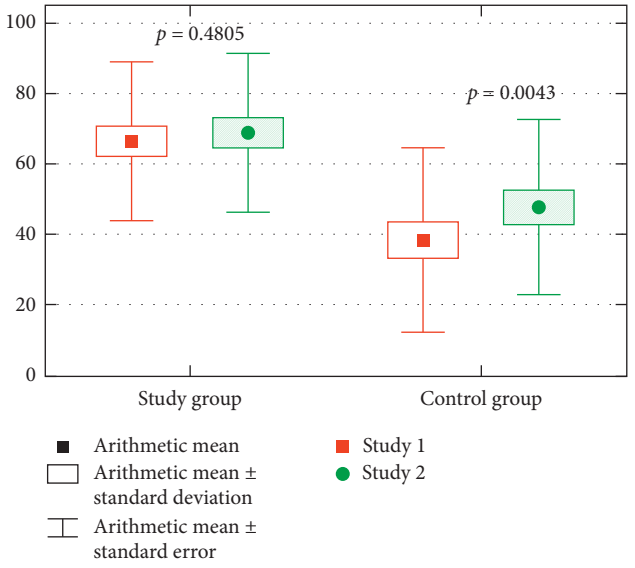

Figure 3: The API values in both groups of patients in study I and study II.

trimethoprim. Amoxicillin with clavulanic acid is not recommended for infections with bacteria from this group. Among Gram-negative aerobic bacteria, gentamicin and ciprofloxacin were those medications that affected all cultured species, for which 100.00% of strains were found to be susceptible, as well as amoxicillin with clavulanic acid (16.67% of susceptible strains). The remaining drugs recommended for bacterial infections of this group included biseptol (95.00% of susceptible strains), cefuroxime (90% of susceptible strains), cefepime (80% of susceptible strains), and ampicillin (3.33% of susceptible strains). According to the EUCAST guidelines, in the case of aerobic infection, the use of natural penicillin, vancomycin, piperacillin with tazobactam, clindamycin, or metronidazole is not recommended. The obtained results of the susceptibility of microorganisms were divided depending on the group (the study group vs. the control group) and are presented in Table 3. Detailed results of the susceptibility of cultured microorganisms divided into four groups (Gram-positive aerobic, Gram-positive anaerobic, Gram-negative aerobic, and Gram-negative anaerobic) are presented in Table 4.

4.3. Assessment of Healing. In the study group, 21 patients showed no complications, and on the follow-up visit on the 10th postoperative day, improvement in general and local condition was noted. Abnormal wound healing which required further procedures was found in 5 patients during the follow-up visit:

(i) 1 patient reported severe pain lasting a week after the extraction of the causative tooth. Finally, after 2 weeks, the pain subsided. The patient did not take antibiotics.

(ii) 3 patients did not report improvement after the purulent lesion incision. Another incision and drainage of purulent content reservoirs at the follow-up visit were necessary. Two of them were

Table 3: Comparison of microbial susceptibility to antibacterial drugs in the study and control groups.

Antibacterial agent		Control group	Study group	p
P	N	27	19	$p = 0.6113$
	Ns	19	12	
	%	**70.37**	**63.16**	
AUG	N	32	27	$p = 0.7517$
	Ns	25	22	
	%	**78.13**	**81.48**	
Va	N	25	19	$p = 0.5375$
	Ns	18	12	
	%	**72.00**	**63.16**	
TZP	N	21	19	$p = 0.5593$
	Ns	17	16	
	%	**80.95**	**84.21**	
CC	N	28	20	$p = 0.1746$
	Ns	27	16	
	%	**96.45**	**80.00**	
MZ	N	25	17	$p = 0.1204$
	Ns	10	11	
	%	**40.00**	**64.71**	
G	N	7	9	$p = 1.0000$
	Ns	7	9	
	%	**100**	**100.00**	
Bs	N	10	9	$p = 0.1238$
	Ns	7	9	
	%	**70.00**	**100.00**	
CXM	N	7	7	$p = 0.5000$
	Ns	7	6	
	%	**100.00**	**85.71**	
CIP	N	7	9	$p = 0.5625$
	Ns	7	8	
	%	**100.00**	**88.89**	
FEP	N	7	7	$p = 0.0962$
	Ns	4	7	
	%	**57.14**	**100.00**	
AM	N	7	7	$p = 0.5000$
	Ns	0	1	
	%	**0.00**	**14.29**	

P, penicillin; AUG, amoxicillin with clavulanic acid; Va, vancomycin; TZP, piperacillin with tazobactam; CC, clindamycin; MZ, metronidazole; G, gentamicin; Bs, sulfamethoxazole with trimethoprim (biseptol); CXM, cefuroxime; CIP, ciprofloxacin; FEP, cefepime; AM, ampicillin.

taking antibiotics—AUG. One patient did not take antibiotics.

(iii) 1 patient suffered a prolonged outflow of purulent exudate and a trismus persisting for 5 days. Seton was removed after 9 days and the treatment was successful. The patient was taking CC antibiotic.

In 81% (21) of patients in this group, palpable submandibular and/or submental painful nodes were found during the physical examination on the first visit. In the control examination after 10 days, the lymph nodes were palpable and painless in 92% of patients (24 out of 26). In the control group, 24 of 26 patients underwent no complications and the healing period was uneventful:

(i) 1 patient was diagnosed with dry alveolus on the follow-up visit after 3 days. The patient visits the clinic the next few days to rinse the alveolus with NaCl physiological solution and to apply the acetylsalicylic acid tablet (Nipas). The patient came to the clinic for the last time 10 days after the surgery, revealing a significant improvement in the local condition. The patient was taking an antibiotic—AUG.

(ii) 1 patient reported severe pain and trismus during the follow-up visit 10 days after the procedure. The patient had control visits after 4 weeks and after 3 months, each time reporting persistent pain, which was gradually reduced. It was only after 6 months that the symptoms completely subsided. The patient was taking an antibiotic—AUG.

Comparing the percentage of specific strains to the occurrence of postoperative complications, it was shown that there is a statistically significant relationship between the incidence of complications and the occurrence of strains of Gram-negative aerobic bacteria ($p = 0.0261$) (Table 5). 5 patients had Gram-negative aerobic strains and postoperative complications: 3 of them were in the study group and 2 in the control group. All patients were undergoing antibiotic therapy: four of them—AUG and one (in the study group)—CC. The cultured species included: *Enterobacter kobei* (1 strain), *Enterobacter cloacae* (2 strains), *Providencia rustigianii* (1 strain), and *Chryseobacterium indologenes* (1 strain). 4 of the 5 strains strained were AUG resistant, and one (*Chryseobacterium indologenes*) was susceptible. The susceptibility of strains to CC was not determined in any of the studied cases (according to EUCAST 8.1, it is not recommended for these cases).

5. Discussion

Literature data show that the microorganisms that cause odontogenic infections include both aerobic and anaerobic bacteria, as well as both Gram-positive and Gram-negative. There is no antibacterial drug that would have such a wide spectrum of activity to effectively counteract all isolated species [18]. The key to success in the empirical antibiotic therapy is to learn which bacterial species are the most common cause of this type of infection, as well as which antibacterial drugs will have the greatest chance of success. In dentistry, the most commonly used antibacterial agents include β-lactam antibiotics (penicillins and cephalosporins), lincosamides (clindamycin), macrolides (azithromycin), fluoroquinolones (ciprofloxacin), and nitroimidazole derivatives (metronidazole) [19–21]. A common characteristic of penicillins and cephalosporin is the β-lactam ring, which, by combining with the penicillin-binding protein (PBP), is responsible for the bactericidal activity of the antibiotic [22]. Natural penicillins, sensitive to β-lactamases, are characterized by a narrow spectrum of antibacterial activity, mainly directed against Gram-positive bacteria. Penicillins with an extended spectrum of activity exhibit a much wider spectrum of antibacterial activity. They

Table 4: Distribution of susceptibility in individual groups of bacteria depending on the antibacterial drug.

		Antibacterial agent																																			
		P				AUG			Va			TZP			CC			MZ			G			Bs			CXM			CIP			FEP			AM		
Species	*N*	*n*	Ns	%	*n*	Ns	%	*n*	Ns	%	*n*	Ns	%	*n*	Ns	%	*n*	Ns	%	*n*	Ns	%	*n*	Ns	%	*n*	Ns	%	*n*	Ns	%	*n*	Ns	%	*n*	Ns	%
Gram-positive anaerobic																																					
Actinomyces naeslundii	17	17	12	70.59	17	17	100.00	17	11	64.71	17	15	88.24	17	15	88.24	17	13	76.47	0	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0	NA
Actinomyces odontolyticus	4	4	3	75.00	4	4	100.00	4	3	75.00	4	4	100.00	4	4	100.00	4	0	0.00	0	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0	NA
Propionibacterium propionicum	4	4	2	50.00	4	4	100.00	4	3	75.00	2	1	50.00	4	4	100.00	4	0	0.00	0	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0	NA
Clostridium perfringens	3	3	3	100.00	3	3	100.00	3	3	100.00	3	3	100.00	3	3	100.00	3	0	0.00	0	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0	NA
Actinomyces israelii	3	3	2	66.67	3	3	100.00	3	2	66.67	2	1	50.00	2	2	100.00	2	0	0.00	0	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0	NA
Clostridium sporogenes	2	2	1	50.00	2	2	100.00	2	0	0.00	2	0	0.00	2	1	50.00	2	2	100.00	0	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0	NA
Actinomyces meyeri	2	2	2	100.00	2	2	100.00	2	2	100.00	2	2	100.00	2	2	100.00	2	0	0.00	0	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0	NA
Clostridium novyi biovar A	2	2	2	100.00	2	2	100.00	2	2	100.00	2	2	100.00	2	1	50.00	2	2	100.00	0	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0	NA
Clostridium butyricum	1	1	0	0.00	1	1	100.00	1	0	0.00	1	1	100.00	1	1	100.00	1	1	100.00	0	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0	NA
Clostridium chauvoei	1	1	0	0.00	1	1	100.00	1	0	0.00	1	0	0.00	1	1	100.00	1	1	100.00	0	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0	NA
Clostridium novyi	1	1	0	0.00	1	1	100.00	1	0	0.00	1	1	100.00	1	1	100.00	1	1	100.00	0	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0	NA
Clostridium tertium	1	1	0	0.00	1	1	100.00	1	1	100.00	1	1	100.00	1	1	100.00	1	1	100.00	0	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0	NA
Actinomyces viscosus	1	1	1	100.00	1	1	100.00	1	1	100.00	0	0	NA	1	1	100.00	1	0	0.00	0	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0	NA
Gram-negative anaerobic																																					
Bacteroides ovatus	1	1	1	100.00	1	1	100.00	1	1	100.00	1	1	100.00	0	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0	NA
Fusobacterium nucleatum	1	1	1	100.00	1	1	100.00	1	1	100.00	1	1	100.00	1	1	100.00	1	0	0.00	0	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0	NA
Gram-positive aerobic																																					
Streptococcus pneumoniae	3	1	1	100.00	0	0	NA	0	0	NA	0	0	NA	3	2	66.67	0	0	NA	0	0	NA	2	0	0.00	0	0	NA	1	0	0.00	0	0	NA	0	0	NA
Staphylococcus aureus	2	0	0	NA	0	0	NA	0	0	NA	0	0	NA	2	2	100.00	0	0	NA	1	1	100.00	2	2	100.00	0	0	NA	0	0	NA	0	0	NA	0	0	NA
Staphylococcus epidermidis	1	1	0	0.00	0	0	NA	0	0	NA	0	0	NA	1	1	100.00	0	0	NA	0	0	NA	1	1	100.00	0	0	NA	0	0	NA	0	0	NA	0	0	NA
Gram-negative aerobic																																					
Klebsiella oxytoca	3	0	0	NA	3	1	33.33	0	0	NA	0	0	NA	0	0	NA	0	0	NA	2	2	100.00	3	3	100.00	3	3	100.00	3	3	100.00	3	1	33.33			
Enterobacter cloacae	2	0	0	NA	2	0	0.00	0	0	NA	0	0	NA	0	0	NA	0	0	NA	2	2	100.00	2	2	100.00	2	2	100.00	2	2	100.00	2	0	0.00	2	0	0.00
Escherichia coli	2	0	0	NA	1	0	0.00	0	0	NA	0	0	NA	0	0	NA	0	0	NA	1	1	100.00	1	1	100.00	1	1	100.00	1	1	100.00	1	1	100.00	1	0	0.00
Klebsiella pneumoniae	2	0	0	NA	2	1	50.00	0	0	NA	0	0	NA	0	0	NA	0	0	NA	2	2	100.00	2	1	50.00	2	2	100.00	2	2	100.00	2	2	100.00	2	0	0.00
Burkholderia cepacia	1	0	0	NA	1	0	0.00	0	0	NA	0	0	NA	0	0	NA	0	0	NA	1	1	100.00	1	1	100.00	0	0	NA	1	1	100.00	1	1	100.00	0	0	NA
Chryseobacterium indologenes	1	0	0	NA	1	1	100.00	0	0	NA	0	0	NA	0	0	NA	0	0	NA	1	1	100.00	0	0	NA	1	1	100.00	1	1	100.00	1	0	0.00			
Enterobacter aerogenes	1	0	0	NA	1	0	0.00	0	0	NA	0	0	NA	0	0	NA	0	0	NA	1	1	100.00	1	1	100.00	1	1	100.00	1	1	100.00	1	0	0.00			
Enterobacter kobei	1	0	0	NA	1	0	0.00	0	0	NA	0	0	NA	0	0	NA	0	0	NA	1	1	100.00	1	1	100.00	1	1	100.00	1	1	100.00	1	0	0.00			
Hafnia alvei	1	0	0	NA	1	0	0.00	0	0	NA	0	0	NA	0	0	NA	0	0	NA	1	1	100.00	1	1	100.00	1	1	100.00	1	0	0.00	1	0	0.00			
Providencia rustigianii	1	0	0	NA	1	0	0.00	0	0	NA	0	0	NA	0	0	NA	0	0	NA	1	1	100.00	1	1	100.00	1	0	0.00	1	1	100.00	0	0	NA	1	0	0.00
Serratia odorifera	1	0	0	NA	1	0	0.00	0	0	NA	0	0	NA	0	0	NA	0	0	NA	1	1	100.00	1	1	100.00	1	1	100.00	1	11	100.00	1	1	100.00	1	0	0.00

N, number of strains of a given microorganism cultured; *n*, number of strains for which susceptibility to a given antibiotic has been determined; Ns, number of strains shown to be susceptible to a given antibacterial agent; %, percentage of bacteria susceptible to a given antibacterial agent; NA, no indications to assess the effect of a particular antibacterial agent on the test microorganism (according to the EUCAST 8.1 guidelines); P, penicillin; AUG, amoxicillin with clavulanic acid; Va, vancomycin; TZP, piperacillin with tazobactam; CC, clindamycin; MZ, metronidazole; G, gentamicin; Bs, sulfamethoxazole with trimethoprim (biseptol); CXM, cefuroxime; CIP, ciprofloxacin; FEP, cefepime; AM, ampicillin.

Table 5: Comparison of the percentage of individual groups of bacteria to the occurrence of complications in the postoperative period.

Bacteria	Total number of patients ($n = 52$)	Postoperative complications		p
		Not present ($n = 45$)	Present ($n = 7$)	
Gram-positive anaerobic	48.97% (25/52)	46.67% (21/45)	57.14% (4/7)	0.9128
Gram-negative anaerobic	3.85% (2/52)	4.44% (2/45)	0.00% (0/7)	0.6259
Gram-positive aerobic	11.54% (6/52)	13.33% (6/45)	0.00% (0/7)	0.6956
Gram-negative aerobic	28.85% (15/52)	22.22% (10/45)	71.43% (5/7)	0.0261
Gram-positive anaerobic vs. Gram-negative anaerobic, p		<0.0001	0.0350	—
Gram-positive aerobic vs. Gram-negative aerobic, p		0.2728	0.0105	—
Gram-positive anaerobic vs. Gram-positive aerobic, p		0.0006	0.0350	—
Gram-negative anaerobic vs. Gram-negative aerobic, p		0.0136	0.0105	—

act on all microorganisms susceptible to natural penicillin, as well as a number of other pathogens responsible for the development of paramaxillary infections, including *Haemophilus influenzae*, *Haemophilus parainfluenzae*, *Escherichia coli*, and *Proteus mirabilis*. However, there are still many strains resistant to their activity, including those belonging to *Enterobacter*, *Citrobacter*, *Klebsiella*, or *Pseudomonas aeruginosa* species [23]. Certain β-lactamases induced upon the growth of multidrug-resistant (MDR) strains with antibiotics are important in conferring resistance to antibiotics [24]. At this point, β-lactamase inhibitors, e.g., clavulanic acid, should be mentioned. Their use allows the deactivation of the majority of β-lactamases produced by Gram-negative bacteria, including *Enterobacter* spp., *Klebsiella pneumoniae*, or *Pseudomonas aeruginosa* mentioned above. The combination of the already effective amoxicillin with the β-lactamase inhibitor creates a mixture with a huge spectrum of antibacterial activity and a broad therapeutic potential [25]. Cephalosporins are similar in terms of their activity. Oral cephalosporin III, available in the oral form (e.g., cefuroxime axetil), is widely used in outpatient dental surgery, demonstrating effects on bacteria that often cause odontogenic infections, including *Streptococcus* spp., *Staphylococcus* spp. (except MRSA), and *Haemophilus influenzae* (also for strains resistant to penicillin) [26, 27]. In addition, they have good permeability to bone tissue and relatively high resistance to β-lactamases [28]. Another type of activity is demonstrated by the widely used lincomycin derivative, clindamycin. By connecting to the 50s ribosomal subunit of a bacterial cell, it inhibits the elongation of the polypeptide chain, which is the basis of its bacteriostatic activity [29]. This drug is effective against bacteria that cause odontogenic inflammation, including *Staphylococcus* spp. (also MRSA), *Streptococcus* spp., *Prevotella melaninogenica*, *Fusobacterium* spp., *Mycoplasma pneumoniae*, and *Clostridium perfringens*. It is also characterized by excellent penetration into bone tissue and hard dental tissues [30]. Clindamycin is not effective in the case of i.a. *Pseudomonas aeruginosa*, and it is also not very potent in infections caused by Gram-negative aerobic bacteria; hence, it is recommended to combine it with third-generation cephalosporins (e.g. cefuroxime). This combination provides a broad spectrum of activity against the majority of Gram-positive and Gram-negative bacteria, both aerobic and anaerobic [25]. Unfortunately, this antibiotic induces a strong dysbacteriosis of the gastrointestinal tract, which in 10–20% of patients may be the cause of persistent diarrhoea, and in combination with the presence of the *Clostridium difficile* strain in the intestines, it is responsible for the occurrence of pseudomembranous colitis [31, 32]. Among the derivatives of nitroimidazole, metronidazole is often used as a bactericide by blocking the synthesis of DNA within a bacterial cell. It is a drug that works particularly well in anaerobic conditions—anaerobic and relatively aerobic bacterial environment, as well as anaerobic protozoa. Bacteria showing a high degree of sensitivity to metronidazole include *Veillonella* spp., *Fusobacterium* spp., *Prevotella* spp., *Peptococcus* spp., *Clostridium* spp., and above all the *Clostridium difficile* species. It should not be combined with bacteriostatic clindamycin. Its combination with amoxicillin or cefuroxime is common and effective [25]. Mücke et al. [14] examined 205 patients diagnosed with perimandibular abscesses and divided them into two groups. The first one was subjected to intraoral incision of the lesion under local anaesthesia, immediately after the patient reported the symptoms. In the case of the remaining patients, the lesions were incised extraorally in general anaesthesia. The necessity to prepare the procedure, including anaesthetic consultation, in each case delayed the implementation of the treatment. In the first group, it was more often necessary to perform repeated surgical procedures (including a second, extraoral incision). However, in these patients, better wound healing effects were observed, together with fewer inflammatory complications ($p < 0.00001$), and the average duration of hospital stay was shorter than in the second group ($p = 0.049$). There was also a positive correlation between the healing effects and the use of amoxicillin with clavulanic acid, which the authors recommend as a first-line drug in the case of a perimandibular abscess. This study proves that in the case of purulent inflammations in the head and neck area, the key element of treatment is the elimination of their source (removal of the causative tooth), as well as the drainage of purulent content (incision). The most important factor affecting the outcome of the treatment is its fast implementation [14]. Orzechowska et al. [33], when analysing the bacterial flora present in odontogenic inflammatory changes, noted a significant predominance of Gram-positive bacteria (74.5%) in comparison with Gram-negative (24.4%) microorganisms. The most common bacteria were *Streptococcus mitis* and *Streptococcus oralis*. Significant immunisation of Gram-positive organisms tested for all antibacterial agents over the period of 5 years was

noticed. The highest increase in microbial resistance was observed in the case of ampicillin and imipenem [33]. In the present study, significant resistance of the cultured bacteria to ampicillin was also found (only one strain of *Klebsiella oxytoca* was found to be susceptible in all examined cases, which is 7.14% of the tested bacteria). All the strains, however, turned out to be susceptible to imipenem. Similar studies conducted by Sobottka et al. [34] showed that 98% of strains cultured from odontogenic inflammatory changes appeared to be susceptible to moxifloxacin and 96% of strains to amoxicillin/clavulanic acid. Clindamycin was effective in 60% of the studied microorganisms [34]. Rams et al. [35] were investigating the sensitivity of bacterial flora in chronic periodontitis and reported the presence of drug-resistant strains in 74.2% of studied patients, among whom 55.0% had strains resistant to doxycycline, 43.3% of patients had strains resistant to amoxicillin, and 26.5% of patients had clindamycin-resistant strains [35]. A lot of research has been conducted to explain the desirability of prophylactic antibiotic therapy in healthy patients before and/or after ITM removal [36]. The positive effect of the drug used on postoperative healing is proven [36–43]. At the same time, many authors show a lack of legitimacy of prophylactic antibiotic therapy, citing a number of negative effects of its abuse [44–47]. Gbotolorun et al. [45] were investigating the group of patients receiving amoxicillin and metronidazole after tooth extraction and found the presence of inflammatory complications in 16% of individuals, compared to 12% in the placebo control group [45]. Xue et al. [44] examined the quality of wound healing after the removed ITM depending on the perioperative antibiotic use. The study was conducted on 207 patients, each of them had a total of 2 ITM removed during 2 visits. In all cases, one treatment was carried out with the use of an antibiotic (amoxicillin or clindamycin, from 1 hour before the surgery to 3 days after the procedure). In the second group, placebo was used instead of an antibiotic. There were no statistically significant differences in postoperative wound healing, neither did any inflammatory complications occur [44]. In contrast to previous investigators, López-Cedrún et al. [48] showed that the use of antibiotic (amoxicillin) significantly affected the postoperative pain and the incidence of complications, i.e., postoperative wound infection, trismus, fever, or dysphagia. In addition, it was shown that the best effects in preventing complications after the removal of ITM were obtained by using a postoperative antibiotic [48]. Schüssl et al. [30] examined the concentration of antibiotics (amoxicillin and clindamycin) in dental hard tissues after oral administration for 60–120 minutes before extraction. The observed concentration of antibiotics exceeded the MIC90 value for some potentially pathogenic microorganisms present in the oral cavity, which confirms the validity of using these drugs, especially in the case of heavier and more vulnerable ITM removal procedures [30]. The abuse of antibiotics in dentistry is a problem known all over the world [49, 50]. This phenomenon is strictly related to the formation of multidrug-resistant strains of bacteria and causes complications in many different branches of medicine [12, 24, 51]. Marra et al. [20] showed that although in the years 1996–2013 the total frequency of prescribing antibiotics by physicians fell by 12.77%, at the same time the frequency of prescribing antibiotics by dentists increased by 62.2% [20]. In the Czech Republic, the frequency of prescribing amoxicillin and clindamycin increased by 60% in the years 2006–2012 [52]. In Germany, amoxicillin and clindamycin are also the most frequently prescribed antibiotics by dentists. In 2015, they were prescribed in 45.8% and 31.7% of all cases, respectively [53]. Also in Poland, the number of administered antibiotics is constantly increasing. Detailed studies carried out by Chlabicz et al. [54] show that in 2004–2008, over 50% of patients treated with antibiotics used penicillins, in particular amoxicillin, alone or with the addition of β-lactamase inhibitors [54]. The probable causes of the abuse of antibiotics by dentists are frequent errors in the treatment of odontogenic inflammation (antibiotic therapy instead of causative treatment), but also the slow adaptation to the latest recommendations, limiting the use of antibiotics in patients with cardiac defects, population aging, or popularisation of dental implants and related complications [35, 50, 55]. To sum up, the most important aspect of an effective treatment of odontogenic inflammation involves the correct diagnosis and immediate surgical intervention, with the antibiotic aspect being of secondary importance [56]. The broad spectrum of activity and the relatively low risk of side effects favour the use of penicillin. The benefits of using clindamycin are associated with its excellent penetration of bone tissue, which is the focus of odontogenic inflammation. Unfortunately, often the only action taken by dentists in cases of the development of acute inflammation is antibiotic therapy without the implementation of a surgical procedure. This is inconsistent with the modern medical knowledge and exposes the patient to a number of serious potential complications [57]. Treatment should be preceded by a thorough medical interview, and it should be tailored individually to each patient. Numerous evidence points to the low effectiveness of prophylactic therapy in healthy people [45, 47]. It should be considered whether the potential benefits outweigh the risk of adverse effects [58]. The conclusions based on the results of the planned testing can be practically used during the updating and possible modification of the recommendations regarding empiric antibiotic treatment used both in patients with acute oral inflammatory conditions and in patients after elective surgery in an outpatient procedure.

6. Conclusions

(1) Differences in susceptibility of cultured bacterial flora were found, depending on the type of the bacteria. Among the anaerobic bacteria, the highest percentage of susceptible strains was found for amoxicillin with clavulanic acid and clindamycin. Among the aerobic bacteria, the highest number of bacterial strains was found to be susceptible to gentamicin, ciprofloxacin, and cefuroxime.

(2) In cases of odontogenic inflammation, the primary treatment should be implementation of an appropriate surgical procedure. In the presence of systemic symptoms, it seems reasonable to use an additional combination of antibiotics (amoxicillin with cefuroxime or ciprofloxacin or clindamycin with cefuroxime) to provide a broad spectrum of antibacterial activity.

(3) The procedure of removal of the third impacted molar leads to a decrease in oral hygiene during the first week after its implementation, which was proven by a statistically significant increase in API tested immediately before and a week after surgery.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors certify that they have no affiliations with or involvement in any organisation or entity with any financial or nonfinancial interest in the subject matter or materials discussed in this study.

Authors' Contributions

Mateusz Bogacz and Tadeusz Morawiec authors have contributed equally to this work.

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Research Article

Gold Standard Evaluation of an Automatic HAIs Surveillance System

Beatriz Villamarín-Bello,[1] Berta Uriel-Latorre,[1] Florentino Fdez-Riverola,[2,3,4] María Sande-Meijide,[1] and Daniel Glez-Peña[2,3,4]

[1]*Preventive Medice Service, Complexo Hospitalario Universitario de Ourense, Rúa Ramón Puga 52-56, 32004 Ourense, Spain*
[2]*Department of Computer Science, University of Vigo, ESEI—Escuela Superior de Ingeniería Informática, Edificio Politécnico, Campus Universitario As Lagoas s/n, 32004 Ourense, Spain*
[3]*CINBIO—Biomedical Research Centre, University of Vigo, Campus Universitario Lagoas-Marcosende, 36310 Vigo, Spain*
[4]*SING Research Group, Galicia Sur Health Research Institute (IIS Galicia Sur), SERGAS-UVIGO, Vigo, Spain*

Correspondence should be addressed to Daniel Glez-Peña; dgpena@uvigo.es

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Hospital-acquired Infections (HAIs) surveillance, defined as the systematic collection of data related to a certain health event, is considered an essential dimension for a prevention HAI program to be effective. In recent years, new automated HAI surveillance methods have emerged with the wide adoption of electronic health records (EHR). Here we present the validation results against the gold standard of HAIs diagnosis of the InNoCBR system deployed in the Ourense University Hospital Complex (Spain). Acting as a totally autonomous system, InNoCBR achieves a HAI sensitivity of 70.83% and a specificity of 97.76%, with a positive predictive value of 77.24%. The kappa index for infection type classification is 0.67. Sensitivity varies depending on infection type, where bloodstream infection attains the best value (93.33%), whereas the respiratory infection could be improved the most (53.33%). Working as a semi-automatic system, InNoCBR reaches a high level of sensitivity (81.73%), specificity (99.47%), and a meritorious positive predictive value (94.33%).

1. Introduction

Hospital-acquired (nosocomial) infections are defined as infections contracted in a hospital environment being not present, nor in the incubation period, at the inpatient admission date [1]. Following this definition, it is commonly accepted that those infections occurring after the first 48 h from the hospital admission date are considered as hospital-acquired infections (HAIs). In Europe, it is estimated that 4,100,000 patients suffer from any type of nosocomial infection every year. In this context, the EPPS (European Point Prevalence Survey) showed that, in the 2011-2012 period, the prevalence of patients with at least one HAI in acute care hospitals was 6.0%, which means that 1 from 18 admitted patients suffer one HAI every day. This prevalence increases to 19.5% in intensive care patients. According to WHO (World Health Organization) data, 37,000 deaths are directly related to HAIs and up to 16 million of avoidable hospital admissions take place in Europe every year.

The surveillance, defined as the systematic collection of data related to a certain health event, is considered an essential dimension for a prevention HAI program to be effective [2, 3]. In such a situation, surveillance activities are a first step towards HAI prevention, showing that through the implementation of appropriate surveillance and control programs, a reduction of up to 20%–30% in the occurrence of HAIs can be achieved [4–6]. As part of this approach, traditional surveillance is based on time-consuming manual inspections, which require (i) daily revision of lengthy lists containing micro-organisms found in positive cultures from the microbiology service and drug prescriptions from the pharmacy service, (ii) regular visits to the medical inpatient units, (iii) revision of the clinical histories (e.g., evolution records, annotations from nursing staff, analytical data, etc.,), and (iv) compute the necessary calculations for estimating the infection rate. All these activities should be done in advance, within a reasonable short time, in order to establish appropriate corrective actions where necessary in the most quick and efficient manner. While the

benefits of this traditional real-time surveillance are undeniable, this mode of operation is expensive and difficult to assume for the vast majority of the preventive medicine services, which often see other relevant activities seriously undermined.

However, new automated HAI surveillance methods have emerged with the wide adoption of electronic health records (EHR). These systems facilitate the daily work of surveillance while, at the same time, improve the effectiveness and efficiency of the whole process, allowing the monitoring of large hospital areas with an optimum use of available resources. For example, Du et al. [7] developed and validated RT-NISS, a real-time automatic hospital-wide HAIs surveillance system in China. The validation over 974 (85 HAI and 889 nonHAI cases) manually checked inpatients gave excellent rates of sensitivity (98.8%) and specificity (93.0%), and a more modest positive predictive value (PPV) (57.53%). They also report time savings against manual review of 200 times. Tvardik et al. [8] studied the feasibility of using Natural Language Processing techniques to automatically detect HAIs in clinical documents. They tested the system over 113 cases (56 HAI and 57 nonHAI cases) and obtained a sensitivity of 83.9% and a specificity of 84.2%. The PPV was not reported, but in a real setting, where the prevalence of HAIs (or proportion of positive cases) is relatively low (about 6%), the reported sensitivity and specificity would lead to a high false discovery rate, or low PPV. A review of automated surveillance of HAIs can be found in [9].

The InNoCBR system is an automatic HAI detection and classification software developed between 2010 and 2013, and is routinely used at the Preventive Medicine Service of CHUO (Ourense University Hospital Complex, Spain), a public hospital belonging to the Spanish National Health System. During all that time, the system was systematically applied to monitor, diagnose, and control HAIs under the supervision of infection control specialists following the well-known surveillance definitions and criteria adopted by the ECDC (European Centre for Disease Prevention and Control). InNoCBR is able to detect and classify HAIs of multiple types including urinary, respiratory, bloodstream, surgical site, cutaneous, enteric, and other type. The system was described and partially validated in [10], but only with those cases that were automatically gathered with an acquisition process inside InNoCBR. In this sense, validation in [10] focused in the ability of InNoCBR to correctly learn from the user (expert) behavior when classifying the correct type of HAI of a suspicious case (by means of Machine Learning techniques). In this sense, in [10] we could not, for example, assess false negatives i.e.,: HAI cases that were not acquired. Moreover, the "gold standard" used in [10] was the InNoCBR user classifications by seeing the patient information gathered by InNoCBR.

Here we present the validation results of the whole InNoCBR system against the gold standard of HAIs diagnosis, i.e., the manual review of every possible case carried out by independent experts.

2. Materials and Methods

2.1. InNoCBR. Taking into consideration that the appropriate identification of HAIs involves the selection of an initial manageable (but highly sensitive) subset of potentially positive cases from the entire patient database, InNoCBR was divided into two well-differentiated operational modules executed by a scheduled task on a daily basis: (i) gathering of potentially positive HAI cases (namely, the acquisition process), and (ii) the intelligent diagnostic module itself. Figure 1 summarizes the InNoCBR architecture and the underlying operational process. The main objective of the first (left side) module is the identification of possible HAI cases irrespective of their location while optimizing sensitivity (i.e., preventing the existence of false negative errors). For its part, the second (right side) module is in charge of executing the intelligent diagnostic process, in which those previous collected cases are classified by infection type taking into consideration evidence found in the hospital information systems.

On the one hand, the acquisition process (left side module in Figure 1) is carried out using two different but complementary sources of information (i.e., databases of microbiology and pharmacy), from which several sets of capturing rules and filters are applied with the goal of discarding those less promising cases. In practice, cases from the microbiology database are selected when positive samples of a certain micro-organism are found in cultures from admitted patients, or patients coming from the emergency department or external consultations. In a complementary action, the pharmacy database is used to find antimicrobial prescriptions with a duration longer than 5 days, selecting those patients as potentially positive HAI cases. Additionally, each single period of hospitalisation may result in several potentially positive HAI cases, as in the case of patient prescriptions being interrupted over one or more days, which are considered as a separate HAI case.

During the application of the set of capturing rules previously commented, different kinds of complementary information (labelled as evidence collection in Figure 1) are also stored for the subsequent phase of filtering and the later execution of the intelligent diagnostic module. This additional knowledge comprises (i) administrative patient data, (ii) surgery done in the prior month analysed, (iii) prosthesis placed in the last year, (iv) hospital admissions in a time window around the analysed period, (v) departments and hospital beds for which a patient goes through including the nursing unit in case of positive micro-organism cultures, (vi) the existence or not of radiological reports, (vii) unstructured nursing comments, (viii) general observations from the microbiology report, (ix) additional annotations about fever, leucocyte presence in urinary samples, and (x) presence of central and peripheral catheters, and/or urinary catheters.

As previously stated, the primary goal of the acquisition process is the identification of all the possible HAI cases (using capturing rules to maximize sensitivity), which usually leads to a large number of potential candidates: many of them generated by the same infection process and the rest being false positives. In order to counteract this situation, InNoCBR applies a filtering procedure which depends on the specific database used as original source of information (i.e., microbiology or pharmacy).

The specific filtering rules used for those cases coming from the microbiology database are the following: (i) positive blood cultures of the same day for a given patient only generate a potentially positive HAI case belonging to the first positive

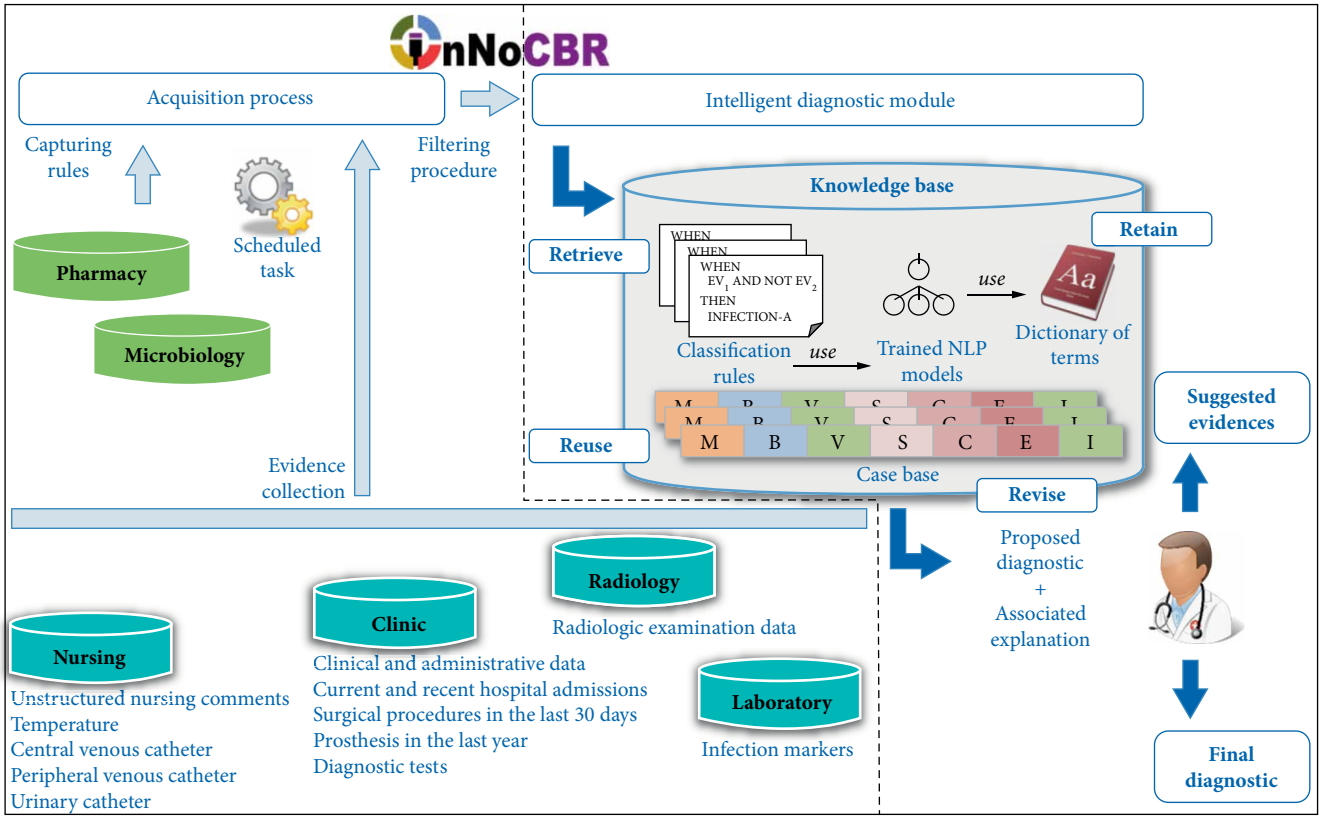

Figure 1: InNoCBR system overview: application architecture and operational process.

blood culture, (ii) positive blood cultures and any other sample related with the same micro-organism in a time window of 4 days (back and forth), only generate a unique potentially positive HAI case linked to the sample which is not of blood, (iii) considering a time window of 10 days, positive cultures of the same sample and the same micro-organism (even if there are more than one micro-organism in the sample) only generate a unique potentially positive HAI case, (iv) positive samples of nasal exudate and catheter tip are not considered as potentially positive HAI cases, (v) positive samples (not related to hospitalisation) belonging to patients who did not undergo surgery in the previous month or prosthesis in the last year, are not taken into account, (vi) positive cultures within the first two days after hospitalisation are not considered unless they are exudate, in which case it will be checked whether the patient underwent surgery or prosthesis, and (vii) positive samples of bronchial suction or bronchoalveolar lavage belonging to outpatients from external consultations are not considered.

In respect of the filtering rules used for those cases coming from the pharmacy database (characterized by the prescription of an antibiotic), InNoCBR takes the following into consideration: (i) if a sample from the microbiology database exists in a time window of 4 days (back and forth) from the day of initiation of the given antibiotic treatment, this case will not be considered as a potentially positive HAI, (ii) samples from the pharmacy database whose day of initiation of the antibiotic treatment falls between the first 2 days of the clinical event will not be considered as potentially positive HAI cases, (iii) samples from the pharmacy database that do not have any radiological report nor positive leucocytes associated in a time window of 4 days (back and forth) from the starting date of the acquisition process are automatically discarded, and (iv) if the difference between the acquisition date of two samples coming from the pharmacy database is 4 days or less, the latter is excluded.

On the other hand, the intelligent diagnostic module (right side in Figure 1) automatically classifies every potentially positive HAI case (gathered in the previous acquisition phase) as one of the following categories: (i) HAI, including its location, (ii) extrahospitalary infection, or (iii) no infection. The development of the intelligent classification model was the result of a previous doctoral thesis titled "*Intelligent system for searching and classification of nosocomial infection cases*" [11] whose main contributions can be found in [10]. In summary, the intelligent diagnostic module is based on a CBR (Case-Based Reasoning) system [12], equipped with (i) a set of manual rules provided by experts focused on urinary, surgical, and bloodstream infection types and (ii) a set of automatic extracted rules (AER) that deal with samples that are not classified by the manual rules that were derived by using the PART Machine Learning algorithm [13]. Additionally, a NLP (Natural Language Processing) unit is able to handle precise electronic physician narratives and daily comments from nursing staff in order to provide additional clues about the occurrence of underlying HAIs.

2.2. Gold Standard. The gold standard for the comprehensive evaluation of the InNoCBR system was based on the collection and storage of data from periodic visits to patients admitted to six selected units, which included both (i) gathering individual comments about the patient follow-up from the nursing staff, and (ii) the direct observation of certain relevant symptoms. All the data gathered by means of these on-site visits were the complement to the information provided through EHR, which included data coming from microbiology results, analytical determinations, imaging studies, physician narratives, and comments from nursing staff, previous admissions and information from primary care services.

Each of the inpatient units participating in the study was analysed during a time frame of one month, although the follow-up time of each individual patient was not equal for all. In case of patients already admitted in the unit during the beginning of the study, the monitoring was carried out from the inpatient admission date to hospital discharge, patient transfer to another unit or the very last day of the study. In addition, those patients who were admitted in the unit once the study was started only were monitored until their hospital discharge, transfer to another unit or the very last day of the study.

Taking into consideration all the information collected and structured during the entire period (i.e., the gold standard), an infection control specialist made a final diagnosis for each analysed patient choosing one of three available categories: (i) HAI, (ii) extrahospitalary infection, or (iii) no infection.

2.3. Experimental Design. The present work is a validation study of a diagnostic test, and therefore a descriptive, comparative, and transversal study was particularly designed to adequately compare the output generated by an autonomous HAIs surveillance system against the gold standard. As in previous related works form the scientific community, standard measures were used to validate the accuracy and overall performance of our InNoCBR system including sensitivity, specificity, and both positive predictive and negative predictive values. Additionally, the prevalence of nosocomial infection was adjusted by taking into account its value from the EPINE (Study of Prevalence of Nosocomial Infection in Spain) in 2012 [14].

With regard to the population under study and the period analysed, there have been studies on all the patients admitted in the following units during the period specified: (i) traumatology unit in April 2013, (ii) internal medicine unit in August 2013, (iii) intensive care unit (ICU) including in September 2013, (iv) nephrology unit in February 2014, (v) general surgery unit from the 19th March to 20th April 2014, and (vi) reanimation unit from 19th March to 20th April 2014.

In reference to inclusion and exclusion criteria, all the patients admitted in the units mentioned above during each analysed period were included. As a result, a large number of patients were selected with the goal of representing the whole spectra of infection. Throughout the period analysed, some patients suffered a HAI while they were hospitalised while others had no symptoms of infection. In a complementary way, some patients have been excluded from the present study because they were admitted to different units or they did not fulfil the time criterion of the target units.

Table 1: 2×2 confusion matrix for a two-condition prediction system evaluation (TP = true positive, FP = false positive, FN = false negative, TN = true negative).

Prediction system	Gold standard	
	Positive	Negative
Positive	TP	FP
Negative	FN	TN

In relation to the population size, a lower limit was not previously established but a representative set of hospital units were initially selected in order to cover a balanced representation of the disease spectrum over a sufficient period of time. In practice, this resulted in a total of 890 patients distributed as follows: 221 patients from the traumatology unit, 169 patients from the internal medicine unit, 104 patients from the ICU, 43 patients from the nephrology unit, and 353 patients from the general surgery and reanimation units, which were studied as a whole because after a surgical intervention, the patient often remains in the reanimation unit for some time.

With respect to evaluation measures, they can be derived from confusion matrixes. For a prediction system for two conditions (for example discriminating between HAI and nonHAI cases), a 2×2 confusion matrix as the one shown in Table 1 is used, where there can be four types of results: true positives (TP) which are positive cases predicted as such, false positives (FP), which are negative cases predicted as positives, false negatives (FN) which are positive cases predicted as negative, and true negatives (TN) which are negative cases predicted as such.

From this matrix, the sensitivity (Se), as the proportion of positive cases correctly identified, specificity (Sp), as the proportion of negative cases correctly identified, positive predictive value (PPV), as the of positive predictions that are correct and negative predictive value (NPV), as the proportion of negative predictions that are correct, can be calculated with the following formulas:

$$\begin{aligned} \text{Se} &= \frac{\text{TP}}{\text{TP} + \text{FN}}, \\ \text{Sp} &= \frac{\text{TN}}{\text{TN} + \text{FP}}, \\ \text{PPV} &= \frac{\text{TP}}{\text{TP} + \text{FP}}, \\ \text{NPV} &= \frac{\text{TN}}{\text{FN} + \text{TN}}. \end{aligned} \tag{1}$$

For a prediction system that classifies cases between C different conditions (for example classify the correct type of HAI), a $C \times C$ confusion matrix as the one shown in Table 2 can be calculated. Each i, j cell contains the count of cases presenting condition j predicted as having condition i.

The Cohen's kappa index is calculated given a $C \times C$ confusion matrix by the following formula:

$$K = \frac{\sum Xii - \sum XiXi}{1 - \sum XiXi}, \tag{2}$$

where i takes values from 1 to C.

For cases where the true conditions in the obtained gold standard have a different distribution, that is, the real

Table 2: $C \times C$ confusion matrix for a multiple-condition prediction system.

Prediction system	Gold standard							
	1	2	3	4	5	…	C	Total
1	X_{11}	X_{12}	X_{13}	X_{14}	X_{15}	…	X_{1C}	X_1
2	X_{21}	X_{22}	X_{23}	X_{24}	X_{25}	…	X_{2C}	X_2
3	X_{31}	X_{32}	X_{33}	X_{34}	X_{35}	…	X_{3C}	X_3
4	X_{41}	X_{42}	X_{43}	X_{44}	X_{45}	…	X_{4C}	X_4
5	X_{51}	X_{52}	X_{53}	X_{54}	X_{55}	…	X_{5C}	X_5
…	…	…	…	…	…	…	…	…
C	X_{C1}	X_{C2}	X_{C3}	X_{C4}	X_{C5}	…	X_{CC}	X_C
$\sum$	$X_{.1}$	$X_{.2}$	$X_{.3}$	$X_{.4}$	$X_{.5}$	…	$X_{.C}$	n

population prevalence differs from the sample observed one, some measures need to be adjusted for real prevalence. Prevalence-adjusted PPV and NPV values are calculated with the following formulas (prev = population prevalence):

$$\text{PPV} = \frac{\text{Se} * \text{prev}}{\text{Se} * \text{prev} + (1 - \text{prev}) * (1 - \text{Sp})}, \quad (3)$$
$$\text{NPV} = \frac{\text{Sp} * (1 - \text{prev})}{\text{prev} * (1 - \text{Se}) + \text{Sp} * (1 - \text{prev})}.$$

In the multiple-condition case, the adjustment is done by redefining the C × C confusion matrix values as:

$$\text{adjusted}\left(X_{ij}\right) = \frac{X_{ij}}{\sum_{i=1}^{C} X_{ij}} \cdot \text{prev}_j, \quad (4)$$

where prev_j is the true prevalence for condition j.

3. Results and Discussion

Four different sides of InNoCBR were evaluated, including (i) the InNoCBR acquisition process, as the capability of detecting HAIs regardless from its concrete type of infection, measuring sensitivity, specificity, PPV, and NPV, (ii) the InNoCBR intelligent diagnostic process, as the capability to correctly classify those suspicious HAI cases coming from the acquisition module, measuring sensitivity, specificity, PPV, NPV, as well as, Cohen's kappa index for the concrete type of infection agreement, (iii) the global combination of the previous two modules, evaluating the whole automatic HAI detection and classification system as a whole, measuring sensitivity, specificity, PPV, NPV, as well as, Cohen's kappa index for the concrete type of infection agreement, and (iv) the performance as a semi-automatic system, that is, the comparison of the final decision of the InNoCBR user against the gold standard, instead of the InNoCBR's diagnostic proposal.

3.1. Gold Standard. Gold standard fieldwork resulted in a valuable final raw data set comprising 938 possible HAI cases belonging to the 890 patients that conform the population under study in the analysed period. Each patient was identified by its own medical record number, which was subsequently used to review all the information derived from the inpatient hospitalisation with the goal of reaching a consensual diagnosis (i.e., HAI and particular type of infection, extrahospitalary infection or no infection). Table 3 shows the absolute number of cases comprising the gold standard by each analysed unit and type of infection.

From Table 1, as would be expected, it may be observed that the greatest number of surgical site infections (S) occur in the general surgery and reanimation units. Even though, it is also notable the number of urinary infections in these same units, that might be related to the vesical catheterization used by patients in the first hours our days postoperatively.

Additionally, it is also noted that urinary infections are the most common in all the hospital units analysed, which is in line with the study of prevalence of nosocomial infection in Spain (EPINE), being justified by the frequent use of vesical catheters in admitted patients. In respect of respiratory infections, a large number of occurrences is observed in the ICU, which is very probably linked to the inpatient population admitted in this unit, almost all of them requiring mechanical ventilation or being in close contact witch patients with respiratory infections.

The validation of the InNoCBR system compared with the gold standard is addressed as a validation study of a diagnostic test and, therefore, it is of special interest to obtain accuracy and overall performance results in terms of validity and security. In this line, standard measures were used to validate the system including sensitivity, specificity, positive predictive value, negative predictive value, and kappa for its two operational modules (i.e., acquisition process and intelligent diagnostic) working separately and together. In this regard, Table 4 presents the global confusion matrix summarizing the classification results obtained following the experimental design protocol previously commented.

Interestingly, the global confusion matrix presented in Table 4 contains the number of those cases not acquired by the InNoCBR acquisition module (indicated in parentheses). As an example, it may be observed that there are a total of 78 urinary infections, from which 10 are not acquired by the InNoCBR acquisition module, while the remaining are correctly classified as urinary (60) or incorrectly classified as negative by the intelligent diagnostic module. Moreover, it may also be observed that numerous discrepancies take place when a specific type of infection is classified as a negative case, except for two infections of type "O" that were classified as surgical infections by InNoCBR.

3.2. InNoCBR Acquisition Module. As previously commented, the acquisition process of InNoCBR is carried out using different information coming from the microbiology and pharmacy databases, with the goal of gathering potentially positive HAI cases. This section presents the results obtained by the system with regard to its ability to detect actual HAI cases, irrespective of their location. In this line, Table 5 summarizes the results obtained by the InNoCBR acquisition module when compared with the gold standard.

The results obtained show an acceptable level of sensitivity (88.76%), which is the main objective of the acquisition module (i.e., preventing the existence of false negative errors).

Table 3: Gold standard descriptive analysis (U = Urinary infection, R = Respiratory infection, B = Bloodstream, S = Surgical site infection, C = Cutaneous infection, E = Enteric infection, O = Other type of infection, No/Ex = No infection or extrahospitalary infection).

Hospital unit	Type of infection								
	U	R	B	S	C	E	O	No/Ex	HAIs/Σ
General surgery and Reanimation	34	11	8	33	—	2	2	285	90/375
Internal medicine	14	4	1	—	1	—	1	152	21/173
Nephrology	4	—	—	—	1	—	—	38	5/43
Traumatology	12	2	2	9	—	1	1	200	27/227
ICU	14	13	4	3	1	—	—	85	35/120
Σ	78	30	15	45	3	3	4	760	178/938

Table 4: Global confusion matrix InNoCBR VS gold standard with different types of infection(U = Urinary infection, R = Respiratory infection, B = Bloodstream, S = Surgical site infection, C = Cutaneous infection, E = Enteric infection, O = Other type of infection, No/Ex = No infection or extrahospitalary infection). Neg* stands for any classification of InNoCBR different from a HAI: not acquired (indicated in parentheses), ignored, no infection or extrahospitalary infection.

InNoCBR	Infection type (gold standard)								
	U	R	B	S	C	E	O	No/Ex	Σ
U	60	—	—	—	—	—	—	3	63
R	—	15	—	—	—	—	—	1	16
B	—	—	14	—	—	—	—	3	17
Q	—	—	—	40	—	—	2	7	49
C	—	—	—	—	1	—	—	3	4
E	—	—	—	—	—	1	—	1	2
O	—	—	—	—	—	—	—	—	0
Neg*	18 (10)	14 (6)	1 (1)	5 (1)	2 (0)	2 (1)	2 (1)	743 (676)	787
Σ	78	30	15	45	3	3	4	761	938

Table 5: Results obtained from the InNoCBR acquisition module when compared with the gold standard, for a 95% confidence interval.

Sensitivity	Specificity	PPV*	NPV*
88.76% (82.96–92.83)	88.95% (86.45–91.04)	46.26% (39.62–52.63)	98.66% (98.10–99.05)

*Adjusted values for a prevalence of 9.68% (EPINE 2012).

Table 6: Acquired and not acquired HAIs by the InNoCBR acquisition module grouped by infection type (U = Urinary infection, R = Respiratory infection, B = Bloodstream, S = Surgical site infection, C = Cutaneous infection, E = Enteric infection, O = Other type of infection).

InNoCBR	Infection type (gold standard)							
	U	R	B	S	C	E	O	Σ
Acquired	68	24	14	44	3	2	3	158
Not acquired	10	6	1	1	—	1	1	20
Σ	78	30	15	45	3	3	4	178

This value, together with the achieved specificity level and also considering a prevalence value of HAI equals to 9.68%, produces a modest result for PPV (46.26%). This fact highlights the need for the subsequent intelligent diagnostic module, which is in charge of further analysing those previous collected cases to classify true positives by infection type and discard all the false negatives previously collected.

In reference to the acquisition process, in particular when considering the infection type, Table 6 summarizes the results obtained by the acquisition module of InNoCBR.

It is important to note that the number of cases with cutaneous infection, enteric, and other locations is lower than 10, therefore, data are not conclusive in this respect. As for the remainder of the locations in descending order of achieved sensitivity, the following values were obtained: surgical site = 97.78%, bloodstream = 93.33%, urinary = 87.18% and respiratory = 80%. Since the respiratory infection is significantly lower compared to the rest of HAIs, it should be considered as an improvement point of the InNoCBR acquisition process.

3.3. InNoCBR Intelligent Diagnostic Module. Once the acquisition module selects all the potentially positive HAI cases, InNoCBR automatically executes the intelligent diagnostic module. This section presents the results obtained by this module taking only into account those previously acquired cases. Table 7 summarizes the results obtained by the InNoCBR intelligent diagnostic module when compared with the gold standard.

As shown in Table 7, both sensitivity and specificity values are around 80%. PPV is much higher than in the acquisition module, reaching a percentage of 77.24%. Additionally, a moderate kappa value of 0.62 is obtained by the intelligent diagnostic module.

3.4. Global Performance of the InNoCBR System. In order to obtain a comprehensive and aggregate view of the InNoCBR system (i.e., acquisition + intelligent diagnostic modules working as a whole), it can be evaluated following a *black box* approach, without examining each part separately. In this connection, Table 8 shows the same performance measures as before, but considering the system as a whole.

As shown in Table 8, the global sensitivity value of InNoCBR is 70.83%, which is less than the value obtained for the acquisition or intelligent diagnostic modules analysed separately. This can be explained by the fact that some correctly acquired HAI cases, are latter discarded by the intelligent

Table 7: Results obtained from the InNoCBR intelligent diagnostic module when compared with the gold standard, for a 95% confidence interval.

Sensitivity* HAIs	Specificity* HAIs	PPV* HAIs	NPV* HAIs	kappa* index
81.06% (70.38–88.67)	79.76% (69.94–87.09)	77.24% (66.50–85.42)	83.25% (73.57–90.01)	0.62 (0.52–0.71)

*Adjusted values for the following prevalences (EPINE 2012): urinary = 1.54%, respiratory = 1.97%, bloodstream = 1.38%, surgical = 2.61%, cutaneous = 0.31%, enteric = 0.15%, other = 1.72%.

Table 8: Global results obtained from InNoCBR when compared with the gold standard.

Sensitivity* HAIs	Specificity* HAIs	PPV* HAIs	NPV* HAIs	kappa* index
70.83% (60.21–79.6)	97.76% (96.46–98.61)	77.24% (66.50–85.42)	76.00% (72.96–78.80)	0.67 (0.60–0.74)

*Adjusted values for the following prevalences (EPINE 2012): urinary = 1.54%, respiratory = 1.97%, bloodstream = 1.38%, surgical = 2.61%, cutaneous = 0.31%, enteric = 0.15%, other = 1.72%.

diagnostic module. Moreover, in no case the intelligent diagnostic module can improve the acquisition module, because it is impossible to re-acquire any additional HAI case. For its part, the specificity level achieves a 97.76%, higher than the value obtained by the separate modules. The PPV value (77.24%), as would be expected, is the same as in the intelligent diagnostic module and, therefore, significantly higher than the value obtained by the acquisition module. Finally, the Kappa index achieves a moderate value of 0.67.

To complement the global performance study of the InNoCBR system, Figure 2 shows a sensitivity analysis by infection type.

As shown in Figure 2, and omitting those cases with cutaneous infection, enteric, and other locations because of the low number of instances, obtained sensitivities in descending order were bloodstream infection = 93.33, surgical site = 88.89%, urinary = 76.92%, and respiratory = 53.33%. Once again, the respiratory infection should be considered as an improvement point.

With reference to PPV taking into consideration different locations (i.e., reliability of a positive case), Figure 3 shows the results obtained by the InNoCBR system. In this case, the infection labelled as "Other" was not included because there was no such output from the InNoCBR system. As in previous analyses, the low number of cases for cutaneous and enteric locations led to inconclusive results. As for the remainder of the locations in descending order of achieved VPP, the following values were obtained: respiratory = 100.00%, bloodstream = 78.32%, and urinary = 76.87%. Specifically, a high reliability was obtained for respiratory HAIs, because InNoCBR correctly diagnosed all the available cases. However, the number of positive predictions still offers only a limited scope to draw definite.

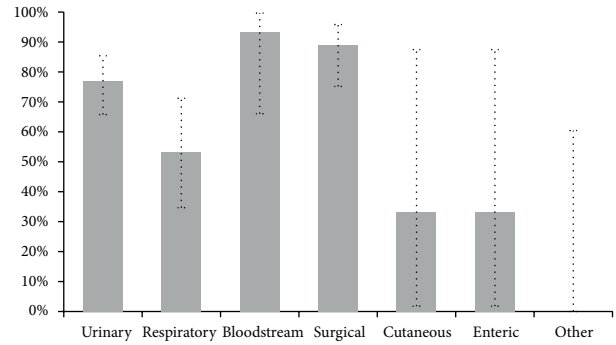

Figure 2: Global sensitivity of InNoCBR when detecting possible HAI cases from different locations. Vertical error bars indicate potential variations with 95% confidence intervals.

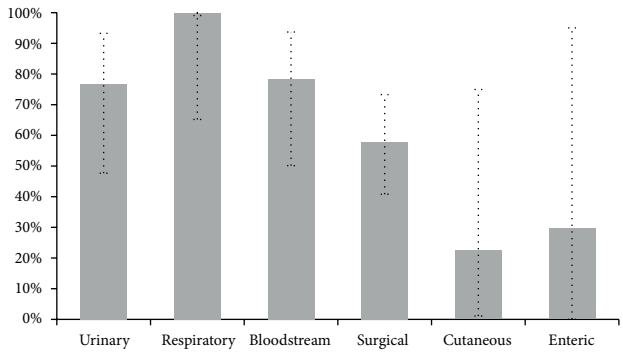

Figure 3: Global PPV of InNoCBR when detecting possible HAI cases from different locations. Vertical error bars indicate potential variations with 95% confidence intervals.

By way of summary, Figure 4 presents the collaborative work between the two InNoCBR modules, working in a co-ordinated manner to obtain results in two key aspects: sensitivity and PPV. In this way, the InNoCBR acquisition module exhibits a high sensitivity percentage at the expense of a high false positive rate (resulting in a low PPV), but the intelligent diagnostic module obtains a much lower false positive rate while keeping an acceptable sensitivity. In this context, it is important to note that the loss in sensitivity shown by the intelligent diagnostic module has a smaller impact than if it would have taken place in the acquisition module, as all the acquired cases are also analysed by the expert, whereas if they are not acquired, they are definitively lost (not been counted).

3.5. InNoCBR Performance Working as a Semi-Automatic Diagnostic System. To complement the validation study, this section analyses the performance of InNoCBR working as a recommendation system for the purpose of assisting the expert with the final diagnosis. In this particular case, the comparison is made between the final diagnostic of the expert (with the assistance of InNoCBR) vs. the gold standard, which can be seen as a global assessment of the Preventive Medicine Service equipped with an automatic tool for the purpose of detecting and classifying HAIs. In this scenario, it must be pointed out that the expert only evaluates those cases previously acquired by InNoCBR. Table 9 presents the new confusion matrix

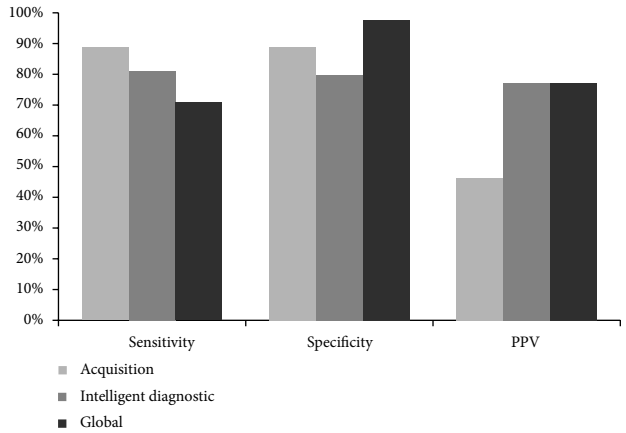

Figure 4: Global sensitivity, specificity and PPV values obtained by the InNoCBR system, disaggregated by the acquisition and intelligent diagnostic modules. Note: both VPP of the intelligent diagnostic module and VPP of the InNoCBR system have the same value by definition.

corresponding to InNoCBR working as a semi-automatic diagnostic system.

On the basis of the confusion matrix introduced in Table 9, Table 10 summarizes the results obtained by InNoCBR working as a semi-automatic diagnostic system when compared with the gold standard.

As shown in Table 10, the global sensitivity value of InNoCBR working as a semi-automatic diagnostic system reaches an 81.73%. This value has a theoretical ceiling of 88.76% derived from the acquisition module of InNoCBR, since the expert does not evaluate any cases that were not previously acquired. In this respect, and as previously mentioned, sensitivity is the measure that should be improved, mainly in the acquisition module. For its part, the specificity level achieves a 99.47%, motivated by the fact that the expert has rectified 80 samples out of 84 negative cases incorrectly acquired. The PPV value attains a 94.33% and the NPV value remains in a 76.00%. Table 8 also includes the Kapa index for those acquired cases that are evaluated by the expert, obtaining an optimum value of 0.91.

To complement this section, Figure 5 shows the sensitivity analysis of InNoCBR working as a semi-automatic diagnostic system by infection type. From Figure 5, and omitting those cases with cutaneous infection, enteric, and other locations because of the low number of instances, it may be observed that obtained sensitivities in descending order were surgical site = 95.56%, Bloodstream = 93.33, urinary = 83.33%, and respiratory = 80.00%. Based on this information, the semi-automatic diagnostic system clearly improves sensitivity values in all cases, and in particular in the respiratory HAI.

Finally, Figure 6 summarizes different performance measures that evidence the improvement achieved by InNoCBR working as a semi-automatic diagnostic system when compared with its intelligent diagnostic module working individually.

3.6. Errors Analysis. Among the 63 errors committed by InNoCBR, there are seven types of discrepancies. In the acquisition process, whose objective is to be highly sensitive, only false negatives can be considered as errors, because a false positive can be corrected by the intelligent diagnostic process that run afterwards. Concretely, a false negative in the acquisition module can be due to (i) lack of acquisition from microbiology or pharmacy databases, (ii) erroneous filtering of a sample acquired from the pharmacy database, or (iii) erroneous filtering of a sample acquired from the microbiology database. With respect to the intelligent diagnostic process, the type of errors include (i) samples acquired from pharmacy are not automatically classified since this feature is not implemented in InNoCBR, (ii) incorrect type of HAI, (iii) false positive, which is a nonHAI that was erroneously predicted as HAI, and (iv) false negative, which is a HAI that was erroneously identified as nonHAI. Table 11 Summarizes these different types of discrepancies found during validation of InNoCBR.

Table 9: Confusion matrix, InNoCBR as a semi-automatic diagnostic system VS gold standard with different types of infection (U = Urinary infection, R = Respiratory infection, B = Bloodstream, S = Surgical site infection, C = Cutaneous infection, E = Enteric infection, O = Other type of infection, No/Ex = No infection or extrahospitalary infection).

User with InNoCBR	Infection type (gold standard)								
	U	R	B	S	C	E	O	No/Ex	Σ
U	65	—	—	—	—	—	—	1	65
R	—	24	—	—	—	—	—	1	24
B	—	—	14	—	—	—	—	1	15
Q	—	—	—	43	—	—	—	—	43
C	—	—	—	—	3	—	—	—	3
E	—	—	—	—	—	2	—	—	2
O	—	—	—	—	—	—	2	1	3
Neg*	13 (10)	6 (6)	1 (1)	2 (1)	0 (0)	1 (1)	2 (1)	756 (676)	781
Σ	78	30	15	45	3	3	4	760	938

Neg*: stands for any classification of InNoCBR different from a HAI: not acquired (indicated in parentheses), ignored, no infection or extrahospitalary infection.

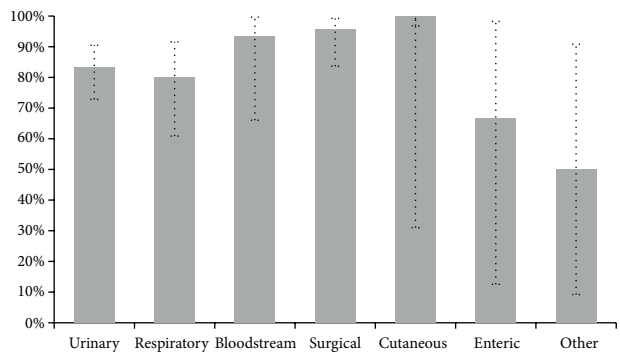

Figure 5: Sensitivity of InNoCBR working as a semi-automatic diagnostic system when detecting possible HAI cases from different locations. Vertical error bars indicate potential variations with 95% confidence intervals.

Table 10: Global results obtained from InNoCBR as a semi-automatic diagnostic system VS gold standard.

Sensitivity * HAIs	Specificity * HAIs	PPV * HAIs	NPV * HAIs	kappa * index	kappa * index (only acquired)
81.73%	99.47%	94.33%	76.00%	0.87	0.91
(71.94–88.77)	(98.63–99.82)	(86.04–98.03)	(72.97–78.79)	(0.80–0.92)	(0.84–0.96)

*Adjusted values for the following prevalences (EPINE 2012): urinary = 1.54%, respiratory = 1.97%, bloodstream = 1.38%, surgical = 2.61%, cutaneous = 0.31%, enteric = 0.15%, other = 1.72%.

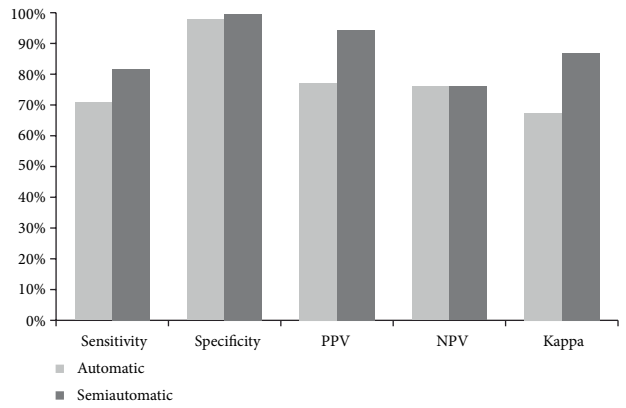

Figure 6: Sensitivity, specificity, PPV, NPV and kappa obtained by InNoCBR (working as an automatic and semi-automatic system) VS gold standard.

As can be observed in Table 11, an important number of errors are produced by erroneous filters (18). We observed that all erroneously filtered microbiology samples were filtered with the following filter (see materials and methods): *positive cultures within the first two days after hospitalisation are not considered unless they are exudate, in which case it will be checked whether the patient underwent surgery or prosthesis*. In those cases a previous surgery or a previous hospitalisation was present, but the sample was not exudate. It could be interesting to consider more different types of samples in this filter rule. We also observed that some erroneously filtered pharmacy samples were due to the following filter: *samples from the pharmacy database whose day of initiation of the antibiotic treatment falls between the first 2 days of the clinical event will not be considered as potentially positive HAI cases*. In some cases, those patients had undergo a recent surgical procedure or admission, so an exception in this sense for this filter could be considered. Another important source of errors is the fact that InNoCBR does not process samples acquired from pharmacy (15 errors), being an evident source of false negatives. Finally, false positives and negatives (17 and 9 errors, respectively) in diagnostic module where produced in samples processed with manually expert-provided rules (15/128 cases) or in samples processed with the automatically extracted rules (13/63). Expert-provided rules have more accuracy than automatically extracted ones, as it could be expected. In this sense, it could be interesting to incorporate manual rules focused on respiratory infections, which showed the lowest sensitivity. However, experts indicated that it would be necessary to access medical comments in RX reports, which was not possible due to technical restrictions related with hospital information systems access.

Table 11: Different types of classification errors found in InNoCBR.

Process	Type of error	Count
Acquisition	False negative	20
	Not acquired	2
	Bad filtering of a pharmacy sample	13
	Bad filtering of a microbiology sample	5
Intelligent diagnostic	Pharmacy samples are not processed	15
	Different type of HAI	2
	False positive	17
	False negative	9

4. Conclusions

Here we have presented the evaluation of an automatic HAI detection and classification system, InNoCBR, which was routinely used at the Preventive Medicine Service of the Ourense University Hospital Complex from 2013. The validation was carried out against the gold standard, where a set of 938 manually reviewed cases where studied (178 HAIs/760 nonHAIs).

Globally, InNoCBR acting totally autonomous present a HAI sensitivity of 70.83% and a specificity of 97.76%, with a good positive predictive value of 77.24%. The kappa index for different type of infection is 0.67. Sensitivity varies depending on infection type, where bloodstream infection presents the best sensitivity (93.33%), whereas the respiratory is the infection type that could be improved the most (53.33%). As a semi-automatic system, taking InNoCBR's user final diagnosis, a high level of sensitivity (81.73%), specificity (99.47%) and, especially, PPV (94.33%) was obtained. This improvement of the semi-automatic evaluation with respect to the totally automatic comes mainly from correcting false negatives in respiratory infections. Moreover, this improvement in the overall accuracy is affordable, since InNoCBR users reported that confirming each new case with the aid of the diagnosis proposal, in combination with the user interface, which gives fast access to key patient information, is relatively fast.

Since 2013, the InNoCBR system has been deployed in more hospitals in Galicia (Spain), and now it is the standard system for HAI surveillance for the Galician public health system. The infection rates decreased from 5.53% (2014) to 4.06% (2018) in CHUO. Periodic reports generated with InNoCBR that are sent to the different medical services and nursing units in a monthly basis, along with comments and improvement proposals, led to this decrease in infection levels

since 2013. Moreover, improvements in the infection prevention bundles have been included for several infection types, such as surgical site, bloodstream, among others. Finally, InNoCBR helped in suggesting changes in the antimicrobial therapy protocols for each type of infection, or even discourage an excessive antibiotic usage. Finally, the intelligent diagnostic module is being continuously auto-evaluated and reports the kappa index of a given period of time. For example, in the year 2018, the kappa index of concordance between the InNoCBR user and the InNoCBR proposals is 0.596, which is similar to that found during validation with 2013 data (kappa of 0.62, see Table 7).

Further directions of InNoCBR improvement could include (i) implementation of intelligent diagnosis for samples acquired in the pharmacy database, (ii) improvements of some filters in the acquisition module, and (iii) improvements of expert-provided, or automatically extracted classification rules.

Data Availability

The gold standard validation data used to support the findings of this study are restricted by the Galician (Spain) Research Ethics Committee in order to protect Patient Privacy.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Hepatitis B Knowledge, Testing, and Vaccination History among Undergraduate Public Health Students in Ghana

Eric Osei,[1] **John Niyilapah**,[2] **and Gregory Kofi Amenuvegbe**[3]

[1]*Department of Population and Behavioural Sciences, School of Public Health, University of Health and Allied Sciences, Hohoe, Ghana*
[2]*Department of Epidemiology, School of Public Health, University of Health and Allied Sciences, Hohoe, Ghana*
[3]*Department of Health Policy, Planning and Management, School of Public Health, University of Health and Allied Sciences, Hohoe, Ghana*

Correspondence should be addressed to Eric Osei; eosei@uhas.edu.gh

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Background. Hepatitis B virus (HBV) infection is a serious public health problem in many parts of the world. The risk of acquiring the infection through exposure to blood, semen, and other bodily fluids is highest among health care workers (HCW) including trainees. Ghana is considered a high risk country for HBV; however little is known about the knowledge and prevention practices of the infection in the country. This study assessed the knowledge, testing, and vaccination history of HBV and their related factors among undergraduate public health students of University of Health and Allied Sciences in Ghana. *Methods*. A cross-sectional study was conducted among 226 students using a pretested questionnaire to assess Hepatitis B knowledge, testing, and vaccination history of the students. We performed logistic regression analysis to examine the relationship between Hepatitis B testing and vaccination history and participants' characteristics. Data was analysed using Stata Version 12. *Results*. Majority 169 (73.9%) of the 226 participants studied had moderate knowledge regarding HBV infection. About half 114 (50.4%) of them had never been tested for HBV infection, and 100 (44.2%) had received at least a single dose of Hepatitis B vaccine. The completed vaccination rate among the students was 30.5%. Students in their 2nd year (Adjusted Odds Ratio [AOR]: 3.13; 95% Confidence Interval [CI]: 1.13, 7.52; p<0.011) and those with moderate (AOR: 4.76; 95% CI; 1.35, 16.82; P=0.015) and good (AOR: 5.40; 95% CI: 1.31, 22.36; P=0.020) level of knowledge were more likely to be tested for HBV. With regard to vaccination, females (AOR: 1.85; 95%CI: 1.04-3.29; P=0.037) and regular students (AOR: 0.37; 95%CI: 0.19, 0.70; p=0.002) were associated with receiving the full dose of Hepatitis B vaccine. *Conclusion*. This study highlights the urgent need for continued health education on HBV infection and strategies that ensure that health trainees are screened and fully vaccinated against the infection to prevent potential future exposure to the virus. The students' representative council can organize free HBV testing and vaccination for all fresh students.

1. Background

The hepatitis B virus (HBV) infection is highly contagious and is transmitted by percutaneous or mucosal exposure to infected blood, semen, or other bodily fluids. Infants born to untreated HBV-infected mothers can also acquire the infection from the mother during birth [1]. The burden of HBV constitutes a public health threat in many parts of the world, despite the availability of an effective vaccine and treatments [2]. According to the World Health Organization (WHO), there were an estimated 257 million people living with chronic HBV infection in 2015 globally, with about 887,000 of them dying from the disease. The burden of HBV infection is particularly higher in sub-Saharan Africa and Western Pacific regions. [3]

Ghana is classified as part of the areas of the world where the burden of HBV infection is considered to be high [4, 5]. Asenso-Ofori and colleagues, for instance, in their systematic review, estimated the prevalence of HBV infection among Ghanaian population to be 12.3% [6]. Similarly, Apama et al. classified Ghana as HBV highly endemic country with an estimated prevalence of 12.92%, a rate higher than that of countries like Cote d'lvoire, Togo, Nigeria, and Burkina Faso [3]. Osei et al. [7] also estimated the prevalence of the

HBV among blood donors to be 7.5% in the Volta region. This suggests that HBV is still a significant public health problem in the country which requires greater attention.

HBV is an important occupational hazard for unvaccinated HCWs including students, with an estimated 2 to 4 times higher incidence than the general population [2, 7, 8]. Knowledge regarding the infection and its related safety precautions among HCWs is therefore essential to minimize the acquired infections in healthcare settings among this vulnerable population as they remain in direct contact with potentially infected persons, blood, and medical tools and instruments during the course of service delivery [9, 10]. Inadequate knowledge of HBV among HCWs, however, may affect their behavioural pattern to vaccination and safety measures.

In spite of the high burden of disease due to chronic Hepatitis B and the improvements and opportunities for care and treatment worldwide, majority of people infected with HBV are unaware of their status and hence normally present with advanced stage of the disease [2]. In the low-income countries, for instance, only about 5% of HBV-infected persons know their infection status. This low uptake of HBV testing is as a result of inadequate testing facilities or services, lack of effective policies and standards for testing, poor laboratory capacity and infrastructure, high cost and complex diagnostic assays and algorithms, and the use of substandard test kits and reagents [2].

The HBV vaccines (HBVc) have been in existence since 1992 and are available as monovalent formulations for birth doses or for vaccination of at risk adult populations [2, 11]. A standard three-dose vaccine regimen, with the second and third doses given 1 month and 6 months apart, respectively, from the initial dose, has been identified as very effective in conferring immunity against HBV [12, 13]. Among HCWs and other healthy adults, HBV vaccination is known to be highly effective for prevention of the infection; hence HBV vaccination has been recommended by the WHO as a primary preventive strategy for the control of the infection among HCWs [14–17].

Many studies have estimated HBV knowledge and vaccination status among HCWs and medical students in many parts of the world [18–22]. Little evidence is however available in Ghana. To bridge the knowledge gap, this study was conducted among undergraduate public health students of University of Health and Allied Sciences, Ghana.

2. Materials and Methods

2.1. Study Design and Setting. A cross-sectional study among undergraduate public health students of University of Health and Allied Sciences was conducted in February, 2017. The University is one of the public institutions in Ghana, established in 2012 to offer higher education to undergraduates and graduates in Health related fields including medicine, Nursing, Midwifery, Pharmacy and other Allied Health Sciences. The University currently has six schools/colleges including the School of Public Health, where this study was conducted. The School of Public Health is located in Hohoe and currently trains undergraduates and graduates in the field of Public Health. The school offers admission to qualified candidates from the Senior Secondary Schools (regular students) into any of the Bachelor of Public Health programs. The school also offers top-up degree programs for healthcare professionals with diploma or certificate in health related fields (top-up students). There are currently about 700 students enrolled in programs such as Disease Control, Health Promotion, Public Health Nutrition, Environmental Health, and Health Information. Master of Public health and Master of Philosophy in Allied Epidemiology are the two graduate courses offered by the school.

2.2. Sample Size and Sampling Procedure. The sample size used for this study was 226 students. This was based on the assumption of 56% of students with adequate knowledge about HBV [23], at 95% confidence level, and 5% margin of error using StatCal option of Epi Info Version 7 and adjusting for a nonresponse rate of 5%. Stratified sampling was used to select the study participants from different year/level of study and different track to ensure representativeness. The number of students selected from each year/level was proportional to the students' population.

2.3. Instrument and Data Collection. The study was carried out using a structured pretested questionnaire written in English. The questionnaire consisted of three parts: (i) demographic characteristics of the respondents; (ii) Hepatitis B testing and vaccination status; (iii) knowledge about Hepatitis B infection. The questionnaire assessed the respondents' general knowledge regarding hepatitis B virus infection, mode of transmission, signs and symptoms, treatment, and preventive measures. The history of HBV testing and vaccination was also assessed through self-reporting. The questionnaires were administered to participants during their free time on the school campus or in their hostels by trained National Service personnel after obtaining informed consent. To enhance data quality and accuracy, the questionnaire was pretested among 20 students in the same school who were excluded from the final study. All inconsistencies and wrong wordings in the questions identified were corrected before the final administration to study participants.

2.4. Data Analysis. The data was entered into Epi Data Version 3.1 for validation and cleaning. Analysis was carried out using Stata statistical package Version 12 (Stata Corp, Collage Station). Descriptive statistics were used to describe the study population in relation to relevant variables. Beyond descriptive statistics, both univariate and multivariate logistic regression models were used to identify factors associated with dependent variables (Hepatitis B testing and vaccination "status") and independent variables. Odds ratio and 95% Confidence Intervals (95%CI) were computed. First, the potential predictors were evaluated for their individual association with the dependent variables in a univariate analysis. Secondly, multiple logistic regression analysis was performed to adjust for possible confounding effect of predictors associated with Hepatitis B testing and vaccination. All predictor variables that showed association with outcome variables ($p < 0.05$) in the univariate analysis were included

in the multivariable model. Vaccination history was modelled as complete vaccination (receiving 3+ doses) and incomplete vaccination (receiving 0-2 doses). The composite measure of students' knowledge was measured by the total number of correct answers to 19 items on knowledge of Hepatitis B. Knowledge was measured using a scoring system where a value of one was assigned to each correct knowledge item and zero for a wrong knowledge item. Knowledge score of 16-19 was considered good knowledge, score of 10-15 was considered moderate knowledge, and a score of less than 10 was classified as having poor knowledge.

2.5. Ethical Issues. This study was ethically approved by the Ethical Review Committee of Ghana Health Service. Permission was sought from the University authorities before data collection. Written informed consent was obtained from each respondent after information about the study have been read and explained to them before interviews were conducted. Confidentiality and privacy were ensured.

3. Results

3.1. Characteristics of the Study Participants. Of the 226 students who participated in the study, 153 (67.7%) were males and the rest females. The ages of the participants ranged from 18 to 42 years with the mean (standard deviation (SD)) age of 24.12 ± 5.01 years. The majority of them 160 (70.8%) were between 20 and 29 years, 195 (86.3%) were never married, and 174 (76.9%) were regular students, while the rest were top-up students, and 181(80.1%) permanently resided in urban areas (Table 1).

3.2. Knowledge on HBV Transmission Dynamics. The mean (SD) knowledge score was 12.98 ± 2.72. Overall, 39 (17.3%) of the students had good knowledge regarding HBV infection. About three-quarters 169 (73.9%) and 20 (8.9%) of them had moderate and poor knowledge, respectively.

With regard to participants' knowledge on HBV transmission dynamics, 145 (65.9%) of them correctly said HBV can be transmitted through unprotected sexual intercourse, 183 (83.2%) knew blood transfusion, and 138 (62.7%) affirmed that people can get HBV infection by sharing towel with an infected person. More than half of the students 125 (56.7%) knew that HBV cannot be transmitted through faeco-oral route and 167 (75.9%) knew that people can acquire the infection by sharing sharps with an infected person, while 171 (53.2%) said HBV is not hereditary. In addition, 140 (63.6%) students knew that people cannot acquire HBV by holding hands with an infected person, and 168 (76.4%) answered correctly that HBV is more infectious than HIV/AIDS, while 161 (73.2%) knew that asymptomatic people can pass on HBV to others (Table 2).

3.3. Knowledge on Signs and Symptoms and Prevention of Hepatitis B. More than half 134 (60.6%) of the respondents knew that HBV-infected persons are asymptomatic at the acute phase. Regarding disease presentation, 127 (57.7%) participants were aware of jaundice as a sign of Hepatitis B infection and 133 (60.5%) knew that HBV can affect other organs order than the liver. Majority 189 (85.9%) of them knew that acute illness due to HBV causes liver inflammation. Concerning treatment and prevention, 154 (70.0%) said HBV infection can be treated and 214 (97.3%) knew that HBV is preventable by vaccination (Table 3).

3.4. Hepatitis B Testing and Vaccination History. About half 114 (50.4%) of the students studied had ever been tested for HBV infection. Of these, 44 (38.6%) got tested less than a year ago, while 70 (61.4%) got tested more than a year prior to this study. Among the students who were never tested for Hepatitis B infection, 82 (36.3%) had no reason for not getting tested, 19 (8.4%) said they could not afford to pay for the test, and 6 (2.7%) did not know where to have the test done. Other reasons given were fear of positive result 4 (1.7%) and did not have time 3 (1.3%). With regard to vaccination, 69 (30.5%) had received 3 completed dose, while 31 (13.7) received incomplete dose (1-2 doses). The rest 126 (55.6%) were never vaccinated against HBV. Regarding the reasons for not been vaccinated, majority 38 (16.8%) of them could not afford to pay for the vaccination, 37 (16.4%) did not have any reason, and 21(9.3%) said they did not receive the vaccine because they were not sick. Other reasons given included did not know where to go for vaccine, did not have time, and did not feel like vaccinating. Two (0.9%) did not receive the vaccine because they were HBV carriers (Table 4).

3.5. Factors Associated with Hepatitis B Testing. In the bivariate analysis, females were 1.26 times more likely to get tested for HBV compared to males, but this did not reach statistical significance. The odds of students who were 30 years and above getting tested was 3.27 times higher compared to students who were less than 20 years (p=0.0497). Students in their third year of study had 3.68 higher odds of being tested compared to their colleagues in their first year of study (p<0.001). Never married students were 42% less likely to get tested compared to those who were married/cohabiting (p=0.028). The likelihood of regular students to get tested for HBV infection was 0.39 times less compared to top-up students (p =0.0033). Students with good knowledge about HBV had 7.14 higher odds of been tested for the infection compared to their colleagues with poor knowledge (p=0.004).

After adjusting for confounding effect of the variables in the multivariate analysis, knowledge level had significant association with HBV testing. Students who had moderate knowledge (OR: 4.76; 95% CI: 1.35, 16.82; p=0.015) and those with good knowledge (OR: 5.41; 95% CI: 1.31, 22.36; p=0.020) were more likely to get tested for HBV compare to their colleagues with poor knowledge. In addition, students who were in their second year of study had 3.13 higher odds of been tested for HBV compared with those in their first year (Table 5).

3.6. Factors Associated with Receiving Full Dose of Hepatitis B Vaccine. In the bivariate analysis, females were 2.18 times more likely to receive complete dose of HBVc compared to

Table 1: Characteristics of study participants.

Characteristics	Frequency	Percentage (%)
Sex		
Male	153	67.7
Female	73	32.3
Age (in years)		
<20	30	13.3
20 - 29	160	70.8
30+	36	15.9
Level of study		
1st year	57	25.2
2nd year	66	29.2
3rd year	59	26.1
4th year	44	19.5
Program of study		
Disease control	96	42.5
Health promotion	18	8.0
Public health general	4	1.8
Environmental health	11	4.9
Health information	40	17.7
Public Health Nutrition	57	25.2
Religious affiliation		
Christian	214	94.7
Muslim	8	3.5
Traditional	4	1.8
Marital status		
Married/co-habiting	31	13.7
Never married	195	86.3
Type of student		
Top-up	52	23.0
Regular	174	77.0
Permanent residential status		
Urban	181	80.1
Rural	45	19.9
Source of funding for education		
Self	43	19.0
Parents	163	72.1
Husband/wife	5	2.2
Other relative(s)	15	6.6

Table 2: Knowledge on causes and mode of transmission of HBV among students.

Knowledge variable	Yes n (%)	No n (%)
Have heard of Hepatitis B infection	220 (97.4)	6 (2.6)
Hepatitis B is cause by a virus	207 (94.1)	13 (6.9)
Hepatitis B virus can be transmitted via unprotected sex	145 (65.9)	75 (34.1)
Hepatitis B virus can be transmitted via Blood transfusion	183 (83.2)	37 (16.8)
Hepatitis B virus can be transmitted via sharing towels with an infected person	138 (62.7)	82 (37.3)
Hepatitis B virus can be transmitted through the air	55 (25.0)	165 (75.0)
Hepatitis B virus be transmitted through the faeco-oral route	95 (43.2)	125 (56.7)
Hepatitis B virus can be transmitted through sharing sharps with an infected person	167 (75.9)	53 (25.1)
Hepatitis B infection is hereditary	103 (46.8)	117 (53.2)
Hepatitis B virus can be transmitted via holding hands with an infected person	80 (36.4)	140 (63.6)
Hepatitis B infection is more infectious than HIV/AIDS	168 (76.4)	52 (23.6)
Asymptomatic Hepatitis B patients can transmit the virus to others	161 (73.2)	59 (26.8)

Table 3: Knowledge on signs and symptoms and prevention of Hepatitis B.

Knowledge variable	Yes n (%)	No n (%)
Infected people are asymptomatic at the acute phase	134 (60.9)	86 (39.1)
Jaundice is a sign of hepatitis B infection	127 (57.7)	93 (42.3)
Acute hepatitis B infection can result in liver inflammation	189 (85.9)	31 (14.1)
Hepatitis B can affect other organs other than the liver	133 (60.5)	87 (39.5)
Hepatitis B infection can be treated	154 (70.0)	66 (30.0)
Hepatitis B infection is preventable by vaccination	214 (97.3)	6 (2.7)

Table 4: Hepatitis B testing and vaccination among respondents.

Variable	Frequency	Percentage
Tested for HBV		
Ever tested	112	49.6
Never tested	114	50.4
Last time got tested for HBV (N=112)		
Less than a year ago	42	37.5
More than a year ago	70	62.5
Reasons for not tested (N=114)		
Do not have any reason	82	71.9
Do not have money	19	16.7
Do not know where to go	6	5.3
Do not have time	3	2.6
Fear of positive test result	4	3.5
HBV vaccine up take		
0 dose (not vaccinated)	126	55.8
1-2 doses (incomplete vaccination)	31	13.7
3 doses (complete vaccination)	69	30.5
Reasons for not vaccinated (N=126)		
Do not have any reason	37	29.4
Do not have money	38	30.2
Do not know where to go	19	15.1
Not sick	21	16.7
Others∗	11	8.7

Others∗ include already a carrier: 2; no need: 3; do not have time: 3; vaccine not available: 1; on medication: 1; and scared of needle: 1.

males (95% CI: 1.13, 4.20; p=0.027). Never married students had 1.78 higher odds of being completely vaccinated compared to those who were married, though not statistically significant. Regular students were about 18% less likely to get fully vaccinated compared to top-up students (95% CI: 0.04, 0.75; p=0.0015). In the multivariate model, being a female showed a significant positive association with complete HBV vaccination (OR: 1.85; 95% CI: 1.04, 3.29; p=0.037) compared with males. Being a regular student (OR: 0.37; 95% CI: 0.19, 0.70; p=0.002) was a predictor for receiving partial or no HBV vaccination (Table 6).

4. Discussion

4.1. Knowledge of Hepatitis B among Undergraduate Students. In this study, we described the knowledge regarding HBV infection, its mode of transmission, signs and symptoms and prevention among Public Health undergraduate students of University of Health and Allied Sciences, Ghana. We also studied the students' HBV testing and vaccination history. It was observed that generally, the majority of students had moderate knowledge regarding HBV infection. Knowledge regarding the mode of transmission among participants is unsatisfactory. Having unprotected sex has been known to be the commonest route of transmission of HBV among adult population [2], however in this study, one-third of respondents disagree to this fact. Similarly, more than 4 in 10 of the respondents wrongly asserted that HBV is transmitted via the faeco-oral route and genes, while nearly half of them said the virus can infect a healthy individual through the air. This clearly indicates that there exist knowledge gap regarding the mode of transmission of the infection. Previous African studies also found knowledge of HBV among medical students and healthcare workers to be unsatisfactory. In

Table 5: Logistic regression analysis of factors associated with HBV testing.

Factors	OR (95% CI)	P-value	AOR (95% CI)	P-value
Sex		0.4218		
Male	1			
Female	1.26 (0.72-2.20)			
Age group (years)		0.0497		
<20	1		1	
20 – 29	1.52 (0.67-3.42)		1.04 (0.38, 2.83)	0.934
30+	3.27 (1.18-9.09)		1.18 (0.25, 5.55)	0.830
Level of study		0.0018		
1st Year	1		1	
2nd year	3.40 (1.61-7.20)		3.13 (1.30-7.52)	0.011
3rd year	3.68 (1.70-7.97)		2.30 (0.88, 6.05)	0.091
4th year	1.96 (0.86-4.46)		1.55 (0.58, 4.14)	0.377
Programme of study		0.6692		
Disease control	1		1	
Health promotion	1.00 (0.37-2.74)			
Mental Health	3.00 (0.30-29.87)			
Environmental Health	0.57 (0.16-2.08)			
Health information	0.74 (0.35-1.55)			
Nutrition	1.19 (0.62-2.30)			
Religion		0.2099		
Christian	1			
Muslim	0.33 (0.07-1.69)			
Traditionalist	3.00 (0.31-29.30)			
Marital status		0.0278		
Married/co-habiting	1		1	
Never married	0.42 (0.19-0.93)		0.94 (0.24, 3.60)	0.924
Type of student		0.0033		
Top-up	1		1	
Regular	0.39 (0.20-0.74)		0.80 (0.23, 2.82)	0.729
Permanent residential status		0.9201		
Urban	1			
Rural	0.97 (0.50-1.86)			
Knowledge level		0.0044		
Poor	1		1	
Moderate	3.95 (1.27-12.32)		4.76 (1.35, 16.82)	0.015
Good	7.14 (1.99-25.59)		5.41 (1.31, 22.30)	0.020

northwest Ethiopia, for instance, 21% of medical students wrongly responded that HBV can be transmitted through the faeco-oral route, while nearly a quarter of them said the infection cannot be transmitted through unprotected sex [24]. In a similar study among Nigerian medical students, Desmond Aroke et al. reported that about 1 in 10 students did not know that HBV can be transmitted via needle stick [22]. Additionally, about 23% of public safety workers did not know the route of transmission of HBV and only 2% identified blood and other bodily fluids as a means through which the virus can be transmitted [25].

This is a worrying trend since HBV infection can pose a great occupational hazard to health workers due to their contact with infected individuals knowingly or unknowingly. Their unawareness regarding the modes of transmission of the infection may expose them to the infection and this could partly explain why HBV is still endemic in this part of the world. Ofori-Asenso and Agyeman in their systematic review estimated HBV prevalence of 12.3% among the general population and attributed this high prevalence to lack of adequate information and understanding of the transmission dynamics of the virus [6]. Among healthy blood donors in the Volta region, Osei et al. observed a seroprevalence of 7.5% and classified it as high-intermediate endemicity [7]. This is a clear indication that HBV infection remains a public health concern in Ghana, hence, the need to strengthen control and preventive measures to reduce the spread of the infection among HCWs and the general population.

In this study, almost half of the respondents answered incorrectly that jaundice is not a sign of HBV infection,

Table 6: Logistic regression analysis of factors associated with complete HBV vaccination.

Factors	OR (95% CI)	P-value	AOR (95% CI)	P-value
Sex		0.0277		
Male	1		1	
Female	2.18 (1.13, 4.20)		1.85 (1.04, 3.29)	0.037
Age group (in years)		0.1775		
<20	1			
20 – 30	0.85 (0.32, 2.30)			
31+	1.03 (0.22, 4.83)			
Level of study		0.8022		
1st year	1			
2nd year	1.48 (0.62, 3.56)			
3rd year	1.40 (0.49, 4.01)			
4th year	1.05 (0.38, 2.94)			
Programme of study		0.5394		
Disease control	1			
Health promotion	0.62 (0.19, 1.97)			
Mental health	0.49 (0.04, 5.29)			
Environmental health	0.22 (0.04, 1.15)			
Health information	1.71 (0.75, 3.92)			
Nutrition	0.67 (0.31, 1.41)			
Religious affiliation		0.5064		
Christian	1			
Muslim	0.31 (0.05, 1.83)			
Traditionalist	1.34 (0.14, 12.56)			
Marital status		0.2028		
Currently married/co-habiting	1			
Never married	1.78 (0.45, 6.95)			
Type of student		0.0015		
Top-up	1		1	
Regular	0.18 (0.04, 0.75)		0.37 (0.19, 0.70)	0.002
Permanent residential status		0.3264		
Rural	1			
Urban	0.66 (0.31, 1.40)			
Knowledge level		0.3264		
Good	1			
Moderate	2.42 (0.63, 9.24)			
Poor	1.82 (0.60, 5.47)			

and more than one-thirds of them were of the view that persons with HBV infection do not remain asymptomatic during the acute phase. These misconceptions regarding HBV disease presentation can influence health seeking behaviour of people with the infection. It has been established that readiness to seek medical care could be potentiated by factors, particularly cues to instigate action such as awareness of disease presentation [26]. Osei et al. reported that Tuberculosis patients without previous knowledge regarding the signs and symptoms of the disease were 5 times more likely to delay seeking medical diagnosis [27]. Expectedly however, Gedefew et al. found relatively high proportion of HCWs being aware that HBV-infected persons may be asymptomatic for a long time [24].

Awareness regarding vaccine as the main preventive measure against HBV infection is considerably high in this study and reached almost universal and is expected to positively influence the students' vaccination attitudes. This result is consistent with other previous studies in Cameroon [22] and Syria [19]. On the other hand, the respondents' incorrect assertion that HBV infection can be cured is a worrying misconception. Similar results were reported in previous study in Kumasi, Ghana [28].

4.2. Testing and Vaccination History among Students. Testing and diagnosis of HBV infection is the gateway for access to both prevention and treatment services and is an essential component of an effective response to the hepatitis epidemic. Testing provides an opportunity to link people to

interventions to reduce transmission, through counselling on risk behaviours and hence the WHO recommends that all adults have routine access to and be offered HBV testing in the general population in settings with ≥2% or ≥5% sero-prevalence of Hepatitis B surface antigen (HBsAg) [1]. However, this current study observed unsatisfactorily low HBV testing rate among the students, results consistent with what was found among Saudi Arabia medical students [18] and among the USA population [20] but lower than what was reported among hospital workers in Nigeria [21]. In Syria, only 16% of medical students knew their HBV status [19]. This is a matter of concern since the prerequisite for HBV vaccination is for one to know his or her status. Hepatitis B testing and vaccination in Ghana outside the Expanded Program on Immunization (EPI) are not covered under the National Health Insurance scheme and hence one has to pay more than a dollar before getting tested. In addition, screenings for HBV are mainly prescribed at hospitals for patients suspected to be reactive to Hepatitis B and blood donors [6]. These could be attributed to the low testing rate among the students observed in this study. Though majority of the students claimed they had no reason for not testing for the infection, a significant proportion (17%) of them said they did not have money to go for the screening. For a country like Ghana, which is classified as a high burden for Hepatitis B to win the battle against the infection, it is important to consider making screening for the infection free of charge for everyone to have access to it irrespective of social class.

HBV vaccination has been recommended by the WHO as primary prevention strategy for the infection among healthcare workers [14]. In this present study, less than one-third of respondents had received 3 complete doses of HBV vaccine as at the time of the study. More than half of them were never vaccinated. This result is similar to what other studies reported [29, 30]. Because of their direct contact with patients or infective materials, HCWs including students are at considerably greater risk of HBV infection than the general population [31, 32] and hence need to be protected against the infection. The cost of vaccination may play a role in the low vaccination rate among the students. In this study, 3 in 10 students were not vaccinated because they could not afford to pay for it. In Ghana, one has to pay more than $4.0 to receive a single dose of HBVc. Poor and incomplete HBVc uptake among health workers and medical students have been reported by many studies [19–22, 25, 29, 32] and the cost of vaccination were cited by most of these studies as the main reason for low uptake.

4.3. Factors Associated with Hepatitis B Testing and Vaccination. Knowledge and level of study were identified in this study as predictors for HBV testing. Students with appreciable level of knowledge about the infection were over 4 times more likely to get tested than those with poor knowledge. This could be because students with good knowledge about Hepatitis B may be much informed on the health threats of the disease and the necessity to get tested to determine their status. The likelihood of HBV test increases with increasing number of years spent in school. This could be as a result of exposure to information about the disease in school since level of education has been found to be increase health awareness and subsequently health seeking.

Sex and been a top-up student were the predictors of receiving full dose of HBVc. Female students are more likely to be fully vaccinated compared to their male counterparts. Thus, females engage more on health seeking behaviour regarding HBV prevention more than males. Males typically are reluctance to consult healthcare providers and the under usage of healthcare service by men have been reported [33]. Ochu et al. observed in their study among safely workers that females had 2.28 times increased chance of receiving full dose of HBVc compared to males [25].

Expectedly, students who are currently not in any employment (regular students) are 37% less likely to be vaccinated against HBV. The employed students (top-ups) are trained health professionals who work in various health facilities in the country and are currently pursuing further education to upgrade their knowledge and skills in their area of specialty, so one can postulate that these category of students may have easy access to the HBVc at their work places and their ability to also afford to pay for the vaccination may account for the difference in vaccination status observed. Unlike this study, a study conducted among adolescents and young adults in Brazil reported a significant association between age and HBV vaccination status [34]. Similar study among dentists in Brazil on the other hand observed that age group and marital status do not have a significant association with HBV vaccination [35].

One obvious limitation of this study is that testing and vaccination status were self-reported and could not be verified by records; hence, recall bias may have an influence on these variables. We however tried to minimize this during data cleaning stage by getting the students to clarify any inconsistent responses.

5. Conclusion

The current study highlights the existence of significant knowledge gap regarding HBV transmission dynamics among the students. Additionally, the low uptake of HBV testing and incomplete or no vaccination among public health students may put them at risk of acquiring the infection. Good knowledge and higher level of study predict HBV testing, while female sex and been a top-up student were positively associated with receiving completed dose of HBVc. The University should therefore consider free screening and vaccination for students in order to protect this vulnerable group from exposure to the virus. Educational programs aimed at improving awareness about the infection are also required.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

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Research Article

Impact of MRSA Transmission and Infection in a Neonatal Intensive Care Unit in China: A Bundle Intervention Study during 2014-2017

Huiping Huang,[1,2] **Jing Ran**,[3] **Jianzhou Yang**,[1] **Peng Li**,[1] **and Guihua Zhuang**[1]

[1]*School of Public Health, Health Science Center, Xi'an Jiaotong University, 76 West Yanta Road, Xi'an 710061, China*
[2]*Department of Infection Control, The First Affiliated Hospital of Xiamen University, 55 Zhenhai Road, Xiamen, 361003, China*
[3]*Department of Gynecology and Obstetrics, The First Affiliated Hospital of Xiamen University, 55 Zhenhai Road, Xiamen, 361003, China*

Correspondence should be addressed to Guihua Zhuang; zhuanggh@mail.xjtu.edu.cn

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Objective. To evaluate the efficacy of bundle intervention on healthcare-associated (HA) methicillin-resistant *Staphylococcus Aureus* (MRSA) infection in the neonatal intensive care unit (NICU). *Methods.* In this study, 11,277 infants having undergone treatment at the NICU in Xiamen, China, from January 2014 to February 2017 were recruited. We retrospectively reviewed patients' demographic and clinical information. Patients from 2014 to 2015 were treated as the control group and those from 2016 to 2017 were classified as the experimental group. Bundle intervention measures were performed, including screening for MRSA, isolation precautions, training of hand hygiene, cleaning protocols, and decontamination of isolation ward. The HA-MRSA data and compliance of infection control measures between both groups were analyzed. *Results.* Through bundle interventions, the compliance with the isolation of MRSA raised from 55.88% to 92.86% and hand hygiene compliance increased from 90.07% to 93.23% ($P < 0.05$). The HA infection decreased from 1.87% to 1.71% ($P > 0.05$) and HA detection rate of MRSA declined from 2.63‰ to 1.00‰, respectively ($P < 0.05$). *Conclusion.* Multifaceted interventions can effectively prevent MRSA infection and transmission; this includes active surveillance, isolation precautions, increased hand hygiene compliance, environmental cleaning, and decontamination.

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one type of multidrug resistant organisms (MDROs) which can remain in the environment for a long term [1]. With the broad-spectrum antimicrobial widely used in clinical settings, incidences of MRSA infection are growing worldwide [2]. MRSA refers to a major healthcare-associated infection (HAI) organism associated with an increasing morbidity and mortality rate. As neonatal immunologic function is immature, MRSA infections occur more frequently in neonatal intensive care units (NICUs). MRSA is reported to be associated with the infection of skin and soft tissue (SSTI) as well as respiratory tracts. In the meantime, HA-MRSA is the main cause of pneumonia, osteomyelitis, and bacteremia [3, 4]. Most neonatal bloodstream infections (BSI) are MRSA-related sepsis which are associated with a high mortality rate [5–7]. HA-MRSA infections are more aggressive and difficult to diagnose and treat which leads to higher mortality rates, longer hospital stays, and increased financial burdens [8, 9].

The prevalence of MRSA is increasing in neonates [10, 11]. MRSA can be spread from environment to patients, and it can also be transmitted by colonized or infected patients [1, 12]. Before a proper cleaning, medical devices (stethoscopes, otoscopes, and thermometers) and various objects in hospital environments were positive for *Staphylococcus* spp., which has been associated with transmission of HAI [1]. MRSA is subject to a higher risk of transmission than MSSA [13, 14]. Control of HA-MRSA remains challenging in NICUs: the recent research identified contaminated or dirty wound operations and MRSA colonization during hospitalization as risk factors for SSI in neonates [15]. Studies demonstrated MRSA had become endemic in numerous NICUs and caused invasive disease and, in some cases, even death [16–18]. For

instance, the majority of outbreaks (in six out of 10) in North London were infected with MRSA [19]. Stricter adherence to disinfection practices by healthcare professionals would help to alleviate outbreak and transmission.

The published research demonstrated that the incidence of MRSA in hospital settings had decreased steadily since 2005 in the US, and this decline may be due to increased attention to infection prevention [20]. Several recent studies also have shown that HA-MRSA decreased significantly after the implementation of the intervention methods [21, 22]. The Society for Healthcare Epidemiology of America (SHEA) has developed guidelines for the prevention of transmission of MRSA within healthcare settings; chief among the recommendations is to improve hand hygiene. Infection control measures were performed to prevent transmission and reduce the risk of acquiring HA-MRSA (e.g., isolation precautions, active surveillance, and hand hygiene compliance). However, whether these measures are effective or sufficient remains controversial [23–25]. Initiative through universal screening and isolation to prevent MRSA infections was considered cost effective [26]; however, universal surveillance results in an overall increase in costs [27]. A patient-centered care bundle intervention is effective but not cost efficient. MRSA are often transmitted through contact with healthcare personnel. The success of an infection control program requires bundle intervention, effective leadership, and a positive work environment. To achieve success, it also relies heavily on the cooperation and participation of healthcare personnel in both behavioral and practice changes [28].

The research studies of the bundle interventions for MRSA are limited, especially in developing countries [17]. The preventability of HA-MRSA in NICU's has rarely been evaluated via in-depth study. Thus, the intervention study was carried out here to explore MRSA transmission and infection trend in NICUs. Patients recruited in this study were from the NICU of a university-affiliated hospital with more than 2000 beds in Xiamen, China. We analyzed the compliance of multifaceted interventions, the clinical characteristics, and the MRSA infection trend. This study aimed to investigate the association of bundle interventions with the MRSA transmission and to provide effective infection control measures to prevent MRSA transmission in NICUs.

2. Materials and Methods

2.1. Setting and Design. The First Affiliated Hospital of Xiamen University is a Joint Commission International (JCI) accredited academic medical center hospital which acquired certification in 2015 and HIMSS EMRAM level 7 in 2017. It houses an 80-bed, Level III NICU. The NICU has 3 private rooms and 4 open bays. 11,277 neonates were admitted to the NICU between January 1, 2014, and December 31, 2017. The relationship between MRSA infection or colonization and these patients was analyzed based on those neonates. During this period, 5,305 neonates were retrospectively reviewed. A bundle intervention study was conducted covering active surveillance, isolation precautions, and hand hygiene promotion, and 5,972 neonates were hospitalized from January 1, 2016, to December 31, 2017.

The collected data, including the medical records of the cases, were extracted from the HAI real-time monitoring system connected with the electronic medical record and inspection system. Medical records were reviewed: this included the demographics, clinical care, microbiologic data, antibiotic resistance, isolation order, and outcomes of the patients with infection or colonization MRSA.

2.2. Surveillance and Cultures. Following the internal University Medicine Goettingen (UMG) guidelines, neonates having high risk factors of MRSA carriers were contacted in isolation, placed in a private room, and then screened for MRSA on admission. The neonate's mother then acts as a potential carrier of the infectious disease and has transferred the infection between hospitals and becomes the high-risk factor for the development of MRSA. Samples of nasal secretions were obtained with a swab for MRSA screened within 24 hours after their admission to the NICU. Bacterial isolation and antimicrobial susceptibility testing were performed in accordance with the methodology of the Clinical and Laboratory Standards Institute [29]. Using the disk diffusion method with S aureus ATCC 25923 as the quality control strain, susceptibility testing was routinely performed. The isolated *Staphylococcus aureus* was cultured with oxacillin at 2μg/ml. If *Staphylococcus aureus* could grow in oxacillin at a concentration greater than 2μg/ml, it was determined to be MRSA.

WHO's (World Health Organization's) "Five Moments for Hand Hygiene" was adopted to assess healthcare workers hand hygiene compliance. Two infection control nurses evaluated hand hygiene compliance though direct observation. The observers remained unchanged during the study. In addition, the contact isolation compliance with MRSA infection or colonization neonates were monitored by infection control nurses. HAIs were verified in accordance with the National Healthcare Safety Network's (NHSN's) surveillance definitions by the Centers for Disease Control and Prevention [30].

2.3. Bundle Interventions. Newborns whose mothers were diagnosed with infection and those transferred from other hospitals were the high-risk factors for the development of MRSA carriers. Accordingly, they would be screened for MRSA. They were placed on contact precautions in a private room following standard precautions until pathogen culture results were reported.

To identify the colonized or infected neonates with MRSA, the doctors in charge should prescribe contact isolation to start the bundle interventions. There are many ways to isolate patients via nurses (e.g., pathogen isolation, alcohol disinfection of the bed, hanging blue contact isolation mark on the door and bed of isolation ward, "MRSA" sign on the patient's electronic medical record, and yellow button on the wristband). Only after all the measures are completed would it be considered a successful intervention. Fractions were used to calculate the intervention success rate. The denominator is the number of patients who should be isolated, and the numerator is the number of patients receiving successful intervention. The medical supplies of

Table 1: Comparison of incidence and resistance rate of MRSA.

Year	Admissions	Neonates with MRSA isolates	Incidence of MRSA ‰	Staphylococcus aureus	Resistance rate of MRSA %
2014	2826	16	5.66	68	23.53
2015	2479	18	7.26	66	27.27
2016	2872	22	7.66	69	31.88
2017	3100	20	6.45	58	34.48

each neonate with MRSA were used solely for that patient (e.g., stethoscope). The bed unit was wiped by nurses with disinfectant three times a day. HCWs (healthcare workers) were trained on proper prevention and how to best control MRSA transmission in a hospital setting, and hand hygiene activities were promoted to improve the hand hygiene compliance of HCWs.

The compliance of contact isolation was supervised by infection control nurses and the implementation was supervised by the infection control professional weekly. The bundle interventions also constructed a supervision mechanism for the medical staff. An official automatic (OA) network would record and report monthly incidents of misconduct where doctors did not prescribe contact isolation advice or nurses did not implement contact isolation. These misconducts were calculated into performance appraisal.

Major monitoring indicators covered the following:

(i) MRSA detection rate of *Staphylococcus aureus*

(ii) The incidence of events of colonization or infection with MRSA neonates

(iii) Rates of healthcare-associated MRSA infections per 1,000 inpatients in NICU

(iv) Rates of community-acquired MRSA infections per 1,000 inpatients in NICU

(v) The hand hygiene compliance of healthcare workers

2.4. Statistical Analysis. In this study, the statistical analyses were performed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA). The continuous variables were presented as mean ± standard deviation (mean ± SD), and the categorical variables were expressed as number and percentage (%). Continuous variables were analyzed using Student's t-test and categorical variables were analyzed using the χ^2 test. Pearson's correlation analysis was performed to investigate the relationship between the rate of HA-MRSA and the rate of contact isolation, HAI, and hand hygiene. All P values were 2 tailed, and $P < 0.05$ was considered as significantly different.

3. Results

3.1. Clinical Characteristics of MRSA Colonized or Infected Neonates. During the study period, 11,277 patients were admitted to the NICU. Among all patients, males made up 54.90%, females made up 45.10%, and 3,383 (30.00%) were either transferred from another hospital or admitted from home. The median length of stay in the NICU was 9.65 ± 10.63 days (range 1-97). 261 neonates (2.31%) had a culture grow *Staphylococcus aureus* and the median age was 15.73 ± 8.05 days. The detection rate of MRSA was 29.12% (76/261) among total isolates of *Staphylococcus aureus*. The median length of stay in the NICU of the neonates with methicillin-susceptible *S. aureus* (MSSA) reached 8.68 ± 8.08 days. MRSA was 14.26 ± 17.91 days. The median length of MRSA was significantly longer than MSSA (t = 2.83, P = 0.005). MRSA infections covered 49 lower respiratory infections (64.47%), 16 skin and soft tissue infections (21.05%), 7 gastrointestinal infections (9.21%), 3 bacteremia, and 1 ventilator-associated pneumonia (VAP). Two of the 76 infected neonates (2.63%) died but only one was due to an MRSA infection. From the periods of 2014-2015 and of 2016-2017, incidents of MRSA in NICUs increased from 6.41‰ (34/5305) to 7.03‰ (42/5972); there was no significant difference ($\chi^2 = 0.16$, $P = 0.686$). The methicillin resistance rate of *Staphylococcus aureus* rose from 25.37% (34/134) to 33.07% (42/127) after implementing bundle interventions; it was not significantly lower ($\chi^2 = 2.68$, $P = 0.102$), as shown in Table 1.

3.2. The Compliance of MRSA Contact Isolation. After implementing bundle interventions the compliance of MRSA contact isolation grew from 55.88% to 92.86% which was significantly higher than before ($\chi^2 = 14.21$, P=0.001), as shown in Table 2.

3.3. The Compliance of Hand Hygiene in NICUs. The compliance of hand hygiene before intervention from 2014 to 2015 reached 90.07% (363/403) and, after intervention from 2016 to 2017, the compliance of hand hygiene rose to 93.23% (909/975). It was significantly higher ($\chi^2 = 4.00$, $P = 0.045$), as shown in Table 3.

3.4. HAI and MRSA Infection of Neonates. The rate of HAI in NICUs dropped from 1.87% to 1.71% after intervention; there was no significant difference ($\chi^2 = 0.41$, P = 0.524). Median length of stay of the neonates with hospital-acquired MRSA after admission was 11.25 ± 6.49 days (range 4-21). The rate of hospital-acquired MRSA after intervention decreased from 2.63‰ to 1.00‰. It was significantly lower ($\chi^2 = 4.24$, P = 0.04). The rate of community-acquired (CA) MRSA increased from 3.77‰ to 6.03‰, therefore displaying no significant difference ($\chi^2 = 2.90$, P = 0.089), as shown in Table 4.

3.5. Isolated Strains of HAI. Among the 201 isolated strains, the main strains included 46 *K. pneumoniae*, 35 *P. aeruginosa*, and 28 *A. baumannii*. The most frequently isolated specimens were sputum (45.27%), blood (22.88%), urine (8.46%), pus (3.98%), etc. The isolated specimen from pus declined from

Table 2: The compliance of MRSA contact isolation.

Year	MRSA Neonates	Neonates in a private room	Ordered contact isolation	Marked as isolation	Number in compliance	Rate of compliance%
2014	16	10	12	12	10	62.50
2015	18	9	11	13	9	50.00
2016	22	20	22	22	20	90.91
2017	20	19	20	19	19	95.00

Table 3: Compliance of hand hygiene in NICUs.

Year	Moments of hand hygiene	Hand hygiene practices	Compliance %
2014	189	167	88.35%
2015	214	196	91.59%
2016	346	318	91.91%
2017	629	591	93.96%

Table 4: HAI and MRSA infection in NICUs.

Year	Admissions	Cases of HAI	Rate of HAI%	MRSA			
				HA		CA	
				Cases	Rate‰	Cases	Rate‰
2014	2826	50	1.77	6	2.12	10	3.54
2015	2479	49	1.98	8	3.23	10	4.03
2016	2872	39	1.36	4	1.39	18	6.26
2017	3100	63	2.03	2	0.64	18	5.81

5.05% to 2.94% after the intervention, and no difference existed in the sequence of main infection sites.

3.6. Correlation between the Rate of Hospital-Acquired MRSA and Contact Isolation Compliance, HAI, and Hand Hygiene Compliance. To find the relationship between the rate of hospital-acquired MRSA, contact isolation compliance, HAI, and hand hygiene compliance, a total of 48 months of data (quarter of a year) were studied. The rate of hospital-acquired MRSA was significantly correlated with contact isolation compliance ($r = -0.888$, $P < 0.01$), and the rate of hospital-acquired MRSA was not significantly correlated with that of HAI ($r = 0.172$, $P = 0.525$) or hand hygiene compliance ($r = -0.311$, $P = 0.241$).

4. Discussion

Some studies have reported that there is a decreasing incidence in MRSA among hospitalized adults in the United States in recent years [31]. Dantes et al. estimated a 54% decline in invasive MRSA [20]. However, MRSA is a common etiological agent of a life-threatening infection in NICUs, with increasing in-hospital mortality rates and prolonging hospital length of stay [3]. In this study, length of stay showed statistically significant differences between MRSA colonized neonates and MSSA colonized neonates and the similar results are shown in the previous report by Geraci et al. [32].

Our study also showed that lower respiratory tract infections with MRSA made up 64.47% while skin and soft tissue infections made up 21.05%. Some studies have reported that MRSA was mostly isolated from blood and bronchoalveolar lavage [3, 33]. Li et al. reported pneumonia (69, 53.1%) was the most common infection of CA-MRSA in Chinese neonates [34]. Of 11,277 neonates, 76 (6.74‰) had a culture grow MRSA including 20 neonates with NICU-acquired MRSA. This was lower than that of other studies which was, respectively, reported as 2% and 5.8% [12, 35].

The annual incidence density of acquisition of MRSA ranged from 5.66 cases to 7.66 cases per 1000 admissions. This was lower than the 6.99% reported by Shirai et al. [3]. Geraci et al. reported that the acquisition of MRSA ranged from a maximum of 20.2 cases for 1000 patient-days to a minimum of 8.8 cases [32]. Infection surveillance is managed by the real-time infection monitor system in hospitals since 2009. The underreporting potential nosocomial infections and MRSA infection were not found in the ward during the study. MRSA infection was lower with the contributing factor being the characteristic difference between western populations and Chinese. The methicillin resistance rate of *Staphylococcus aureus* ranged from 23.53% to 34.48%. HA-MRSA were more likely to develop into severe invasive infections. These infections could even cause death. In this study, one death was attributed to HA- MRSA infection. Harik et al. reported that 68% of MRSA cases were hospital-associated (HA) MRSA [36].

There was a relative risk of 24.2 for colonized patients in NICU to develop an MRSA infection during hospitalization [35]. MRSA contamination from high-touch surfaces is worrisome in developing countries [37]. A bundle of interventions are required in order for NICUs

to prevent MRSA transmission. Single-room isolation is strongly recommended for neonates with high risk factors of MRSA.

Compliance with hand hygiene, active surveillance, and contact isolation either singularly or in conjunction have been insufficient and controversial. One study showed the decrease in MRSA acquisition was primarily attributed to the barrier effects of gowns and gloves followed by improved hand hygiene and lower HCW-patient contact rates [38]. However, other research reported only before and after patient contact rose from 40% to 76% for hand hygiene compliance. HAI and MRSA rates remained high and stable and there was no correlation between compliance and MRSA [25, 39].

A program of universal surveillance, contact precautions, hand hygiene, and institutional culture change was associated with the decrease in healthcare-associated transmissions of and infections with MRSA in an extensive healthcare system [21]. In our study, while the rates of healthcare-associated MRSA infections in NICUs had not changed during the two years before the intervention, they declined under the implementation of the bundle from 2.45 infections per 1000 admissions to 1.17 per 1000 admissions. During the same period, healthcare-associated infections declined slightly from 1.87% to 1.71%. This was because the main infection sites of healthcare-associated infections (including respiratory tract, bacteremia, and urinary tract) and the main pathogens (including *Klebsiella pneumoniae, Acinetobacter baumannii,* and *Pseudomonas aeruginosa)* are Gram-negative conditional pathogens that adapt to the surface of a moist environment and are easy to cause respiratory infection in patients with low immunity. MRSA is a Gram-positive bacterium which makes it easy to colonize on the surface of the human body and spreads through hand contact which is the primary cause of SSTIs.

Accompanied by the implementation of bundle interventions, both hand hygiene compliance and contact isolation grew significantly. Hand hygiene compliance was improved from 90.07% to 93.23% and MRSA contact isolation increased from 55.88% to 92.86%. The major cause of noncompliance was the lack of single room to isolate patients. By analyzing the correlations between the rate of hospital-acquired MRSA, contact isolation compliance, HAI, and hand hygiene compliance, we found that the rate of hospital-acquired MRSA was significantly correlated with that of contact isolation compliance. There was no significant correlation between the rate of hospital-acquired MRSA, HAI, and hand hygiene compliance. This revealed that screening for MRSA in addition to isolation precautions in MRSA bundle interventions are important and effective measures.

5. Conclusions

MRSA bundle interventions are capable of reducing healthcare-associated MRSA infections in NICUs. All research findings show that the bundle interventions are highly effective tools in MRSA prevention and should be adhered to routinely in order to improve contact isolation compliance.

Data Availability

The data used to support the findings of this study are included within the article.

Ethical Approval

This study was approved by the Ethics Committee of the First Affiliated Hospital of Xiamen University.

Disclosure

The funding entities have not influenced the study design, data collection and analysis, or preparation of the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

The Minimum Data Set and Quality Indicators for National Healthcare-Associated Infection Surveillance in Mainland China: Towards Precision Management

Hongwu Yao,[1] Jijiang Suo,[1] Yubin Xing,[1] Mingmei Du,[1] Yanling Bai,[1] Bowei Liu,[1] Lu Li,[1] Rui Huo,[2] Jian Lin,[2] Chunping Chen,[2] Qiang Fu ,[3] and Yunxi Liu [1]

[1]*Department of Infection Management and Disease Control, Chinese PLA General Hospital, Beijing, China*
[2]*HangZhou XingLin Information Technology CO., LTD, Hangzhou, China*
[3]*National Institute of Hospital Administration, Beijing, China*

Correspondence should be addressed to Qiang Fu; mpazy@sina.com and Yunxi Liu; 1425628298@qq.com

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The magnitude and scope of the healthcare-associated infections (HCAIs) burden are underestimated worldwide, and have raised public concerns for their adverse effect on patient safety. In China, HCAIs still present an unneglected challenge and economic burden in recent decades. With the purpose of reducing the HCAI prevalence and enhancing precision management, China's National Nosocomial Infection Management and Quality Control Center (NNIMQCC) had developed a Minimum Data Set (MDS) and corresponding Quality Indicators (QIs) for establishing national HCAI surveillance system, the data elements of which were repeatedly discussed, investigated, and confirmed by consensus of the expert team. The total number of data elements in MDS and QIs were 70 and 64, and they were both classified into seven categorical items. The NNIMQCC also had started two pilot projects to inspect the applicability, feasibility, and reliability of MDS. After years of hard work, more than 400 health facilities in 14 provinces have realized the importance of HCAI surveillance and contributed to developing an ability of exporting automatically standardized data to meet the requirement of MDS and participate in the regional surveillance system. Generally, the emergence of MDS and QIs in China indicates the beginning of the national HCAI surveillance based on information technology and computerized process data. The establishment of MDS aimed to use electronic health process data to ensure the data accuracy and comparability and to provide instructive and ongoing QIs to estimate and monitor the burden of HCAIs, and to evaluate the effects of interventions and direct health policy decision-making.

1. Introduction

Healthcare-associated infections (HCAIs), also known as nosocomial infections, have become an increasingly serious public health issue worldwide because of the effect on morbidity and mortality among hospitalized patients, especially in developing or resource-poor countries [1–4]. The effective prevention and control of HCAI occurrences and transmissions rely heavily on regulatory managements and coordinated interventions [5], which include comprehensive and targeted surveillance, antimicrobial stewardship program, series of infection control bundles, education and training, etc. HCAI surveillance system has been proven to be a powerful tool for estimating and monitoring the national/regional prevalence of infections and evaluating the effect of interventions, and many countries had therefore established national HCAI surveillance systems [3, 6–11], which mainly aimed to develop a Minimum Data Set (MDS) to collect standardized and uniform data about HCAIs from participating healthcare facilities to support data comparison, then to release a Quality Indicators (QIs) for use in benchmarking, public reporting, and pay-for-performance programmes. For example, the US had established a National Nosocomial Infections Surveillance (NNIS) in 1974 using a unified data collection and calculation method and aimed to compare the HCAI rates among different participating

healthcare facilities and departments. And it had been proven to reduce subsequent infection rates by 32% based on an active surveillance programme with feedback to the clinicians and an infection control team [12]. Furthermore, the Danish also had established validate computer-assisted surveillance of HCAI based on selected laboratory and administrative data, which aimed at securing a follow-up on the outcome of the interventions directed at HCAIs [13]. In the last two decades, the system like MONI or TREAT has been made to develop computerized decision support systems to lower the burden of manual HAI surveillance [14]. MONI is filled with patients' administrative and raw medical data. The TREAT system allows for combination of data from different datasets and is also robust to missing data.

In mainland China, the National Institute of Hospital Administration (NIHA) established an HCAI surveillance reporting system decades ago [15, 16]. However, HCAI data was directly and manually reported by participating healthcare facilities. The collected data often lacked validity, resulting in poor data quality, unreliable comparisons, and gaps in the QIs available [17–19]. Although more and more regional health departments and healthcare facilities have started to develop electronic surveillance technology for HCAIs in recent years, the technical structures and data standards in electronic health records (EHRs) differ among healthcare facilities [18, 19]. Health data acquisition is not standardized and uniform at national level so far [20]. In other words, with the development of information technology, the current challenge in mainland China that national electronic HCAI surveillance lacks MDS and corresponding QIs is noticeable [21]. In 2013, the NIHA decided to develop a four-hierarchy system of national-provincial-municipal-institutional Nosocomial Infection Management and Quality Control Centre (NIMQCC) so as to provide an overview of the burden of HCAIs and to improve their management in mainland China [19]. The national NIMQCC (NNIMQCC) was set up, and one of its first steps was to initiate a pilot project and programme to identify MDS with corresponding QIs for a significant HCAI surveillance system at national level.

This study examines the identification and use of the MDS and QIs for national HCAI surveillance in mainland China, to help to address the national HCAI surveillance challenge and emphasize the importance of MDS and QIs in effective data utilization and quality management.

2. Methods

2.1. The Identification of MDS and QIs. Drawing lessons and experiences from both own previous surveillance and other countries' [14, 21–23], the MDS and QIs for national HCAI surveillance should be designed as a practical and efficient tool to focus on the data standard and quality at all stages of reporting process as well as the conclusion interpretations in the feedback reports. The process for developing the MDS and QIs was designed to ensure quality data covering all stages of the surveillance process to allow useful conclusions to be drawn. It therefore focused on directly collecting health process data from electronic health records (EHRs) including hospital information system (HIS), laboratory information system (LIS), electronic medical record (EMR), and radiology information system (RIS). Obviously, the process data was of continuous and traceable characteristics without any artificial errors or optional modifications. For example, we preferred to extract the dates recorded for hospitalization, transfer, and discharge from the EHRs to automatically calculate and acquire the length of hospitalization rather than the result calculated and reported by the HCAI staffs. Then crude data element and Quality Indicator lists were assessed and proposed by both HCAI experts and information technology staffs to ensure that they complied with this requirement. The crude lists were then sent to a multi-institutional and multi-disciplinary expert team including hospital managers, HCAI directors, clinicians, epidemiologists, sterilizing scientists, nurses, and laboratory professors for further improvement and optimization, respectively. Meanwhile, filed trips and inquiries were initiated to investigate both informatization degrees and management challenges in different healthcare facilities. The experts' comments and investigation results were collected and summarized to assess the value and feasibility of each data item and element of MDS and QIs.

2.2. The Development of MDS and QIs. After repeated discussion, investigation, and validation, the final set of items and elements in MDS and QIs were agreed by the expert team. Then the NNIMQCC started two pilot projects for HCAI surveillance to examine the applicability, feasibility, and reliability of MDS and QIs, and the impact of surveillance on the HCAI management and quality control. The pilot projects performed well, and the NNIMQCC carried out a major promotion exercise in 2015 to encourage the establishment of an HCAI surveillance system in more provinces. All healthcare facilities were encouraged to improve their EHRs to ensure that they could provide all the necessary data of HCAI based on MDS. A survey checklist tool with free technical support was created to help participating healthcare facilities to find and solve technical problems with automated extraction and generation of the required data package from EHRs.

3. Results

The data elements of MDS and QIs were both classified into seven categorical items, and the total numbers of the ultimate data elements in MDS and QIs were 70 and 64, respectively. To provide references and explanation for MDS and QIs nationwide, a dedicated book of "guidelines for the implementation of basic data sets and quality control indicators for healthcare-associated infection surveillance" was issued in 2016. Table 1 showed the items in the MDS, covering: basic (12 elements), birth (2 elements), diagnosis (10 elements), treatment (20 elements), microbiology (14 elements), vital signs (4 elements), and HCAI reporting (8 elements). Each data element in the MDS was described by name, numerical code, definition, category, use, correlation indicators, collection, format, permissible values, data source, extraction and description, range of extraction, and scope of exclusion of data elements.

Table 1: Summary of the Minimum Data Set and Quality Indicators for national healthcare-associated infection surveillance.

Data set	Data item	Number of data elements (*n=70*) and indicators (*n=64*)	Data element and indicator examples∗
MDS	Basic	12	Gender, Admission date
	Birth	2	Birth date, Birth weight
	Diagnosis	10	Admission diagnosis, Pathologic diagnosis
	Treatment	20	Operation date, ASA score
	Microbiology	14	Specimen, Antibacterial
	Vital sign	4	Temperature, Diarrhea
	HCAI reporting	8	HCAI type, Outcome
QIs	Infection	6	HCAI rate, Prevalence rate
	Surgical	3	Surgical site infection rate
	Device-related	6	Ventilator-associated Pneumonia
	Neonate	6	HCAI rate in neonate
	Bacterial resistance	10	Multidrug-resistant bacterial infection rate
	Antibiotic use	20	Utilization rate of antibiotics
	On-site-inspection	13	Omission Rate of HCAI

MDS, Minimum Data Set; QIs, Quality Indicators; HCAI, Healthcare-associated Infection; ASA, American Society of Anesthesiologists. ∗Detailed introduction could be found in the dedicated book "Guidelines for the implementation of basic data sets and quality control indicators for Healthcare-associated infections surveillance".

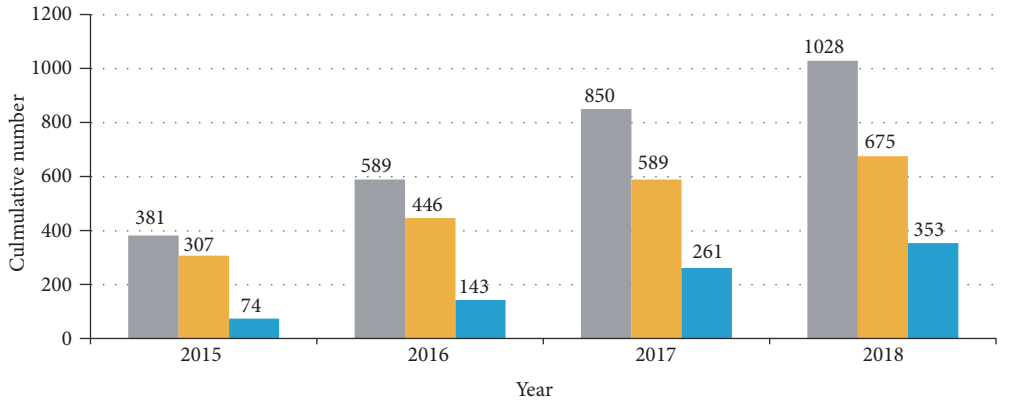
The QIs included various indicators/rates relating to HCAIs, across infection (6 indicators), surgical site (3 indicators), device-related (6 indicators), neonatal care (6 indicators), bacterial resistance (10 indicators), antibiotic use (20 indicators), and on-site inspection (13 indicators). Apart from the indicators about on-site inspection, all could be directly obtained by calculation from the data elements in the MDS. The information about each indicator included its name, numerical code, definition, significance, source, formula, calculation, numerator, denominator, data elements from the MDS, collection, and analysis suggestions.

Two pilot projects were responsible for collecting qualified data packages on HCAIs from healthcare facilities to evaluate HCAI prevalence and provide feedbacks based on MDS and QIs. One pilot project was conducted by Shandong Provincial Hospital at the provincial level and the other was conducted by the Chinese PLA General Hospital at the trans-provincial level. Based on MDS, the data package was based on standard XML format. Data packages from participating healthcare facilities were required to upload every month. The data verification module was set before data submission to make sure that data packages were standardized and qualified. Data was analyzed by the full-time staffs and the feedbacks were mainly consisted of monthly reports, quarterly reports, and annual reports, which were about the HCAI QIs at institutional and regional level. Clearly, the pilot projects provided valuable information about the performance of MDS and QIs and also promoted the national HCAI surveillance system.

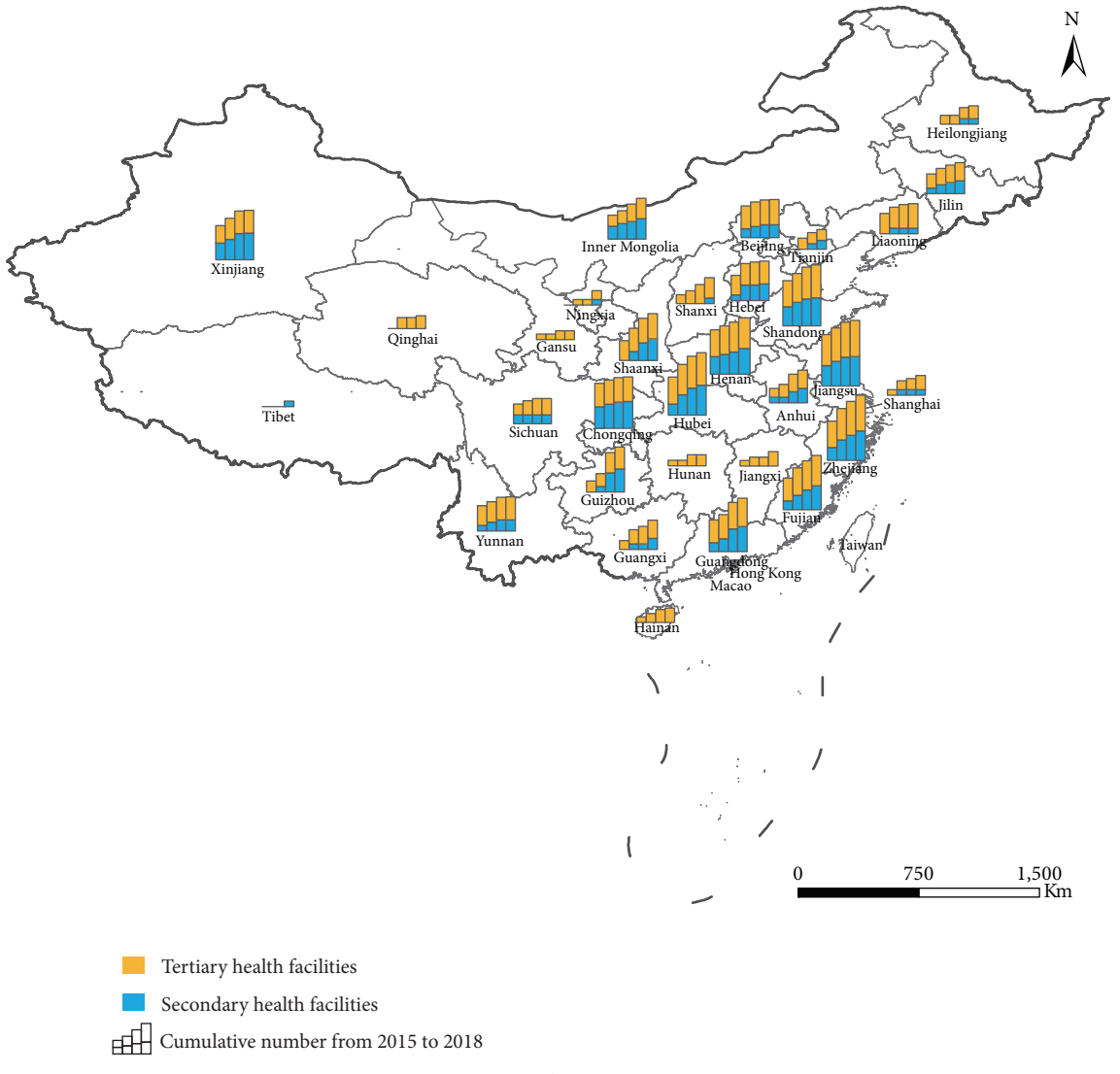
Following encouragement from NNIMQCC and promotion of the pilot projects, more and more healthcare facilities started to prepare for automated extraction of the qualified data package so as to participate in regional and national HCAI surveillance systems. This will allow them to compare their own QIs over time (intrahealthcare facility) and also benchmark against others (interhealthcare facility). These comparisons enable facilities to assess whether their interventions are successful. By September 2018, data from NNIMQCC showed there were one trans-provincial and 13 provincial HCAI surveillance systems established based on the MDS and QIs. These systems were distributed in Beijing (trans-provincial), Shandong, Hubei, Fujian, Zhejiang, Guangdong, Guizhou, Hebei, Yunnan, Shanxi, Inner Mongolia, Ningxia, Shaanxi, and Xinjiang Provinces. In total, about 485 healthcare facilities were involved, of which 200 were providing regular submissions of data packages. It is estimated that more than 1000 healthcare facilities in mainland China had realized the technology requirement for automatic extraction of the qualified data package. As shown in Figure 1, the number has increased steadily since 2015, but there are still far more tertiary healthcare facilities involved than the secondary ones. The number and level of growth varied across provinces, being fewer and slower in Tibet, Qinghai, Gansu, Ningxia, Hainan, Hunan, Jiangxi, Shanghai, and Heilongjiang Provinces.

4. Discussions and Conclusions

Combined use of large data resources and new technologies will solve many existing medical problems and provide better evidence for decision-making in current big data era [24]. Using big data technology to enhance health and medicine is a national priority in China [20]. Data from one health facility is more valid and more effective when it is compared with that from others [14], and up-to-date and accurate data is needed to make precision and informed decisions. The technical structures and data standards in EHRs, however, differ from health facility to health facility in most of countries. To overcome this problem, MDS has been created as a key step for establishment of surveillance system, which has become a tool used by investigators in health services research, outcomes research, and performance improvement to enhance



(a)



(b)

Figure 1: The estimated cumulative number of healthcare facilities with the ability to export the qualified data package from 2015 to 2018 at national (a) and provincial (b) level. The data in 2018 was up to September 2018. The digital province-level map of China was obtained from the Data Sharing Infrastructure of Earth System Science (http://www.geodata.cn) to produce a thematic map of the cumulative number of healthcare facilities in different provinces using ArcGIS 9.2 software (ESRI Inc., Redlands, CA, USA). The significant differences in cumulative numbers across provinces meant that the cumulative number in (b) was adjusted from primary data using a formula: $y = \log_2 (x+1)$.

computerized operation and identify patients with specific conditions and monitor outcomes and process measures [25–27]. For QIs, they were quantitative metric based on MDS and could provide information to improve practice, to monitor performance, to measure achievement, to determine accountability, and to define health policy decision-making [22]. In this study, we has mainly introduced MDS and QIs using for national HCAIs surveillance in mainland China, with the purpose of emphasizing the importance of MDS and QIs in effective data utilization and precise quality management.

It is well known that the HCAI surveillance system could help collect data from participating health facilities to do many data exploration and investigation. Data availability, accessibility, completeness, and validity are imperative for successful implementation of automated HCAI surveillance strategies [28]. The identification of MDS is the most basic task with regard to the data collection in the establishment of automated surveillance system [26], and the useful QIs could help reflect the reality of HCAI prevalence as well as vulnerability of HCAI management. So the availability of national HCAI surveillance with MDS and corresponding QIs is helpful and convenient for HCAI management, as well as for data sharing and comparison on institutional, regional and national level and finally on international level [14].

Although many functioning national HCAI surveillance systems have been built worldwide in recent decades, it is of much imbalance between the developed countries and developing countries [4]. What is especially true is that HCAI surveillance systems at national level are virtually nonexistent in most developing countries. Therefore, not only the health information technology, but also the HCAIs data at national level from developing countries are much scantier than those from developed countries. It is of important and urgency for countries, especially developing countries, to create MDS and QIs, and to establish a significant national HCAI surveillance system as soon as possible. In China, despite significant progress in public health and hospital care, HCAI remains a neglected challenge and economic burden [18, 19, 29]. The under-reporting HCAI prevalence and inadequate medical sources were obvious as well as imbalance healthcare facility development [19]. It was reported that missing report rate from 34.4% of investigated hospitals was greater than 60%, and nearly 50 million of the total 1.3 billion people in China required hospitalization annually because of diseases or trauma, and HCAI was associated with an annual direct economic burden of $1.5-$2.3 billion [29]. Accordingly, NNIMQCC in China focused on the identification of MDS and QIs when it started to develop a national surveillance system to lead and strengthen the precision management of HCAI and contribute to reducing its incidence and prevalence. In order to strengthen the information standardization of HCAI surveillance and solve the problem of standardization of health information in different levels and fields, taking the data elements as the key object in the research, China's Ministry of Health has promulgated a series of standards and regulations [19].

The establishment of the MDS can standardize the data collection in different healthcare facilities, improve the data quality, and ensure the good homogeneity and consistency of HCAI data arrangement and analysis. Another advantage of MDS is that the data is required to be extracted not only in a standard format but also directly from EHRs, which has greatly avoided human participation. Hence, a well-designed information technology infrastructure, data availability, accessibility, completeness, and validity are indispensable [18, 28]. The standardized HCAI data is the process data which is generated and recorded in the EHRs; it not only can better ensure the objectivity and timeliness of the collected data, but also does not need to consume too many resources for the data package exporting and uploading. So the collected data validation and QIs accuracy are guaranteed. The identification of MDS and QIs for national HCAI surveillance in China has already improved data collection, storage, release, and exchange, helping to provide consistent and comparable HCAI data and also enhance decision support for HCAI detection and resource allocation. Also providing a framework for HCAI surveillance, the structured data elements in MDS and QIs can also be of important value to implement models and simulations and other analyses.

The publication of dedicated book has greatly improved the rapid development of HCAI surveillance and supported the beginning of a national HCAI surveillance system based on information technology and computerized process data. Although the increasing trend in different provinces was various (Figure 1). This might be related to the local policies and management of the HCAI surveillance. Despite these variations, however, there is an overall increase in the use of the MDS and QIs in mainland China. In 2018, a few conferences had been organized by NNIMQCC to discuss the further development of national HCAI surveillance system; the value of HCAI surveillance system based on MDS and QIs is recognized. Next, NNIMQCC has planned to introduce related policies and regulations to continuously speed up the establishment of national HCAI surveillance system.

Generally, the HCAI burden in mainland China is increasingly severe and raised public concerns, so the NNIMQCC is responsible for establishing a well unified and definitive MDS and QIs for national HCAI surveillance system to realize the consistency and comparability of HCAI related data, as well as enhancing decision support on HCAI detection and resource allocation. The emergence of MDS and QIs indicated the beginning of the national HCAI surveillance based on information technology and computerized process data. And the publication of the dedicated book has greatly improved the rapid development of informatization in HCAI management. The identification of MDS and QIS for national HCAI surveillance in China has been proven to greatly promote the application of data collection, storage, release, and exchange, so it helps ensure the accurate and effective comparison, statistics and sharing of the information on the prevalence, prevention, and control of HCAIs in mainland China.

Data Availability

This study had only used the data about the yearly number of healthcare facilities during 2015-2018 to describe the

development of surveillance systems established based on the MDS and QIs (Figure 1). This data was from China's National Nosocomial Infection Management and Quality Control Center (NNIMQCC) and contained many detailed information of healthcare facilities (such as name, address, and number of beds). So the data is not publicly available now. About the data availability, readers can contact the author if they have any questions.

Conflicts of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contributions

Hongwu Yao and Jijiang Suo contribute equally to this work.

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Research Article

Recent Advances in Investigation, Prevention, and Management of Healthcare-Associated Infections (HAIs): Resistant Multidrug Strain Colonization and Its Risk Factors in an Intensive Care Unit of a University Hospital

Dana Carmen Zaha,[1] Rita Kiss,[1] Csaba Hegedűs,[2] Rudolf Gesztelyi,[2] Mariann Bombicz,[2] Mariana Muresan,[1] Annamaria Pallag,[3] Miklos Zrinyi,[4] Denes Pall,[4] Cosmin Mihai Vesa,[1] and Otilia Micle[1]

[1]*Department of Preclinical Disciplines, University of Oradea, Faculty of Medicine and Pharmacy, 1st December Square 10, Oradea, 410068, Romania*
[2]*Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, University of Debrecen, Nagyerdei krt. 98, H-4032 Debrecen, Hungary*
[3]*Department of Pharmacy, University of Oradea, Faculty of Medicine and Pharmacy, 1st December Square 10, Oradea, 410068, Romania*
[4]*Coordination Center for Drug Development, Faculty of Medicine, University of Debrecen, Nagyerdei krt. 98, H-4032 Debrecen, Hungary*

Correspondence should be addressed to Rita Kiss; kiss.rita@med.unideb.hu

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Active screening for resistant multidrug strain carriers remains an important component of infection control policy in any healthcare setting indifferent of financial and logistical costs. The objective of our study was to determine the spectrum of bacterial colonization individually among intensive care unit patients. A retrospective observational study was performed in the Intensive Care Unit of Emergency Clinical County Hospital of Oradea during 2017. Medical records of the patients were used for evaluation of source of ICU admission, previous antibiotic therapy, comorbidities, and length of hospital stay. Nasal and groin swabs for MRSA detection and rectal swabs for ESBL, VRE, and CRE detection were collected upon ICU admission of all patients in the first 24 hours and after 7 days. Swab samples were processed for isolation and identification of these resistant multidrug strains. Bacterial colonization on admission was detected in a quarter of patients included in the study. Carbapenemase-producing bacteria were the most common colonizers (21.16%). On admission, 12.06% of patients have been colonized by ESBL-producing members of the family Enterobacterales. Risk factors for colonization on admission to the ICU were chronic liver diseases and chronic renal failure for ESBL infection and chronic liver disease for CRE in male patients. Evaluation of Carmeli's score for male patients showed association only with CRE colonization. Chronic renal failure was found as risk factor for ESBL colonization in female patients. The prevalence of MRSA was 5.23% and less than 1% for VRE. There was no association between any risk factors studied and the presence of *S. aureus* or VRE upon admission. The 7-day ICU stay also proved to be an increased risk for ESBL and CRE infection.

1. Introduction

Infections associated with medical care (nosocomial) represent a worldwide problem despite the advancement in therapeutic technologies [1]. Intensive care unit (ICU) patients are more affected by these infections caused by special pathogens contributing to prolonged intensive care unit stays, increased morbidity and mortality, and increased resource utilization. Special interest is directed at the study of these special microorganisms resistant to multiple antimicrobials,

which are growing at higher rate in the ICU setting, leading to higher treatment costs, morbidity, and mortality [2–6]. An important risk factor for nosocomial infection is prior colonization [7, 8]. Other clinical reports show different data about the incidence and patterns of colonization of multidrug-resistant bacteria [9–11].

As a definition, multiple drug resistance (MDR) is nonsusceptibility of a strain to at least one agent in three or more antibiotic classes [12]. In ICUs, the identified multidrug-resistant microorganisms include both Gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus* spp.) and Gram-negative bacilli (*Escherichia coli*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Proteus* spp., and *Klebsiella* spp.). Methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* spp. (VRE), extended-spectrum beta-lactamase producers (ESBL), carbapenemase-resistant Enterobacteriaceae (CRE), and multidrug-resistant Gram-negative bacteria (MDR) are well described [13–16]. MRSA could be a major cause of severe nosocomial infections (bloodstream infections, urinary tract infections, and pneumonia) contributing to the high mortality because of its resistance to a variety of antibiotics [17]. Vancomycin-resistant *Enterococcus* (VRE) has the same difficulties in treating infections or colonization [18]. Gram-negative organisms are important causes of the same nosocomial infections [19, 20]. ESBL Gram-negative producers are frequently isolated especially in ICU patients with multiple comorbidities and they are associated with high morbidity and mortality, due to the limited therapeutic options [21]. The narrowing of treatment strategies and the limited availability of future underdevelopment drugs lead to spreading of MDR-GN bacterial infections [22, 23]. In recent years, Food and Drug Administration (FDA) has approved the usage of new antibiotic combinations such as ceftolozane/tazobactam and ceftazidime/avibactam that are proven to be effective against MDR-GN, but the emergence of resistant strains is just a matter of time [16, 24]. Antibiotic resistance was discovered with the discovery of antibiotics, and a significant number of the analysed strains isolated from food products showed resistance to different antibiotic classes and the molecular techniques revealed that many of these possess either one or multiple genes that confer resistance to different antibiotics, like presence of ESBL and AmpC production [19, 25].

Bacterial colonization of the skin and mucous membranes of patients with these microorganisms usually precedes infections. Colonizers are part of the commensal bacterial flora of the skin that protects it from colonization with pathogenic bacteria; therefore they do not cause any problems usually. These different colonies may disappear spontaneously or be cleared under the action of disinfectant and antimicrobial agents. However, they can also turn into pathogenic bacteria if the activity of the skin surface antimicrobial proteins (AMPs) is compromised or the strains acquire new genes that modify the disease-producing capacity of the bacteria [26, 27]. When the protective barriers of skin and mucous membranes are compromised or immunity deficiency develops, these organisms overwhelm the body, resulting in an infection [28]. Screening of ICU patients upon admission for colonization by MDR strains, handling them with caution and isolation of colonized patients, could prevent nosocomial infections [29]. The purpose of this screening is to prevent spreading of these organisms to other ICU/non-ICU patients, especially those vulnerable to drastic consequences in case of transmission. A study reported that the screening of ICU patients upon admittance for the presence of MRSA and VRE despite special measures applied to isolate carriers did not result in a reduction of ICU-acquired infection with the aforementioned pathogens [29]. Instead, a recent review of literature published by Glick et al. implies that screening to universal MRSA carriage upon admission may reduce the risk of MRSA infections but the power of evidence was weak to support screening programs [30].

There are several scoring system models in use to estimate the severity of critical illness and to predict mortality among ICU patients. The acute physiology and chronic health evaluation (APACHE) model was first introduced, followed by APACHE II and APACHE III including data from American hospitals [31]. Another model, the simplified acute physiology score (SAPS), was instituted in 1985, followed by the revised editions SAPS II and SAPS III as an alternative to the APACHE score. SAPS I is employing 14 of the original 34 parameters used in the APACHE system and SAPS II and SAPS III included data of 12 parameters from the first 24 hours in the ICU [32–34].

In patients who have severe comorbidities and/or are immunocompromised, the multiple hospitalizations and the long-term antibiotic therapy sometimes in combination represent a high risk in the selection and the colonization and infection with resistant or multidrug-resistant strains [35]. The expected chance with which the bacteria might be resistant to the antimicrobial treatment can be assessed using Carmeli's score [36–38].

This study was undertaken to evaluate the prevalence and the spectrum of bacterial colonization in patients admitted to the ICU of the Emergency Clinical County Hospital of Oradea, and to assess the predisposing risk factors for colonization.

2. Materials and Methods

2.1. Data Collection. The study was conducted at Emergency Clinical County Hospital of Oradea, with a 45-bed ICU that receives patients from within the hospital as well as ones referred from outside. It manages approximately 5600 critically ill patients annually. After ethical clearance by the institution and after receiving informed consent from the patient or from the next of kin (in case of unconscious patient), an active surveillance of 1971 adult admitted patients was done between January and December 2017.

Demographic characteristics, source of ICU admission, previous hospitalization, previous antibiotic therapy, comorbidities, length of hospital stay, and outcome were recorded and analysed. In order to identify the risk factors for colonization with multidrug-resistant strains, the diagnostics have been grouped and analysed as follows: respiratory (pneumonia, bronchial asthma, and chronic obstructive

pulmonary disease) and cardiovascular diseases (hypertension, myocardial infarction, chronic heart failure, coronary heart disease, chronic atrial fibrillation, and stroke), chronic renal failure and diabetic nephropathy, chronic liver diseases (chronic hepatitis, hepatic cirrhosis, hepatic encephalopathy, and chronic hepatic insufficiency), cancer, hematologic malignancy (lymphoma and leukaemia), immunosuppressive status (chronic obstructive pulmonary disease, organ transplant, chronic autoimmune diseases, and AIDS), and diabetes mellitus.

Medical history of the patients, results of physical examination, and multiple laboratory parameters were analysed to calculate Carmeli's score in order to identify patients susceptible to be colonized with multidrug-resistant bacteria at the beginning of the hospitalization. The scoring and stratification were based upon the presence of Carmeli's risk factors [36, 37]. Risk factors were ranked with 1, 2, or 3 points according to the prediction for infection with susceptible, resistant, or multidrug-resistant microorganisms. Patients were scored as 1 to 3 according to the severity of the following factors: the degree of contact with the healthcare system and invasive procedures; antibiotic treatment; and patient characteristics such as age, various comorbidities, or immunosuppressed status. The highest numeric value of the three criteria represents final value of Carmeli's score (1, 2, or 3). The final score allowed us to classify patients as follows: score 1 (community-acquired infections with microorganisms susceptible to classic antibiotics), score 2 (probably healthcare-associated or community-acquired infections but with high probability of resistant or multidrug-resistant strains), and score 3 (maximum prediction for nosocomial infections with resistant or multidrug-resistant strains) [38].

2.2. Microbiological Procedures. Nasal, groin, and/or rectal swab samples were collected to assess bacterial colonization during the first 24 hours of ICU admission from all study participants. The same samples were collected 7 days after admission for patients who remained hospitalized for that time period. Nasal and respective groin swab samples were collected by wiping the swab in circular motion while simultaneously rotating it in 360° and exerting gentle pressure on the surface. Rectal samples were collected by inserting the swab 1 cm into the rectum while rotating the swab in 360°. Swabs collected were placed in sterile round bottom tubes containing 1 ml sterile Amies transport medium and immediately transferred to the laboratory for analysis. Collected nasal and groin samples were screened for MRSA, while rectal swabs were screened for VRE, ESBL, and/or CRE. The strains have been considered multidrug-resistant if they displayed resistance to more than three antibiotic classes. Patients were considered to be colonized by any of the organisms studied if one or more of those organisms was/were isolated from one or more of the swabbed sites.

2.2.1. MRSA. Nasal and groin samples collected were cultured on MRSA CHROMagar (CHROMID® MRSA, bioMérieux) and incubated at 37°C for 24 hours. Plates were examined for green MRSA colonies; other colours were disregarded. In addition, the latex agglutination test was performed to identify penicillin-binding protein, PBP2A. The isolates were confirmed as MRSA by disc diffusion test using 30 μg cefoxitin disc on Mueller-Hinton agar according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. Quality control was done by using *Staphylococcus aureus* ATCC 25923.

2.2.2. VRE. Rectal swab samples were cultured on selective chromogenic medium (CHROMID® VRE) for direct identification of *E. faecalis* and *E. faecium*. The medium allowed the detection of acquired vancomycin-resistant strains (vanA and vanB) by selective coloration of the colonies as follows: blue-green for *E. faecalis* and violet for *E. faecium*. For quality control *E. faecalis* ATCC 51299 was used.

2.2.3. ESBL. All the rectal swabs were processed for isolation of ESBL-producing GN bacteria. Gram-negative bacteria were isolated on ESBL chrome agar (CHROMID® ESBL, bioMérieux). After incubation for 24 hours, it is easy to read results based on the specific coloration. *Escherichia coli* shows a pink to burgundy coloration (ß-glucuronidase-producing colonies), *Klebsiella*, *Enterobacter*, *Serratia*, and *Citrobacter* display a green/blue to brownish green coloration (ß-glucosidase-producing colonies), *Proteus*, *Providencia*, and *Morganella* appear in dark to light brown colonies (deaminase-expressing strains), and *Acinetobacter* spp. shows a cream coloration. ESBL producers were confirmed phenotypically by the double-disk synergy test using clavulanic acid and third-generation cephalosporins. Disks of third-generation cephalosporins and amoxicillin-clavulanic acid were kept 15–20 mm apart, centre to centre, on inoculated Mueller-Hinton agar. The plates were incubated at 35°C–37°C for 18–24 hours. A clear extension of the edge of the inhibition zone of any of the third-generation cephalosporins towards the amoxicillin-clavulanic acid disk was interpreted as positive for ESBL production. *Escherichia coli* ATCC 25922 was used for quality control.

2.2.4. CRE. The rectal swabs were analysed as well for the presence of GN bacteria with a reduced susceptibility to most of the carbapenem agents. We used selective chromogenic medium for the screening of carbapenemase-producing Enterobacteriaceae (CHROMID® CARBA). *Escherichia coli* displays a pink to burgundy coloration, *Klebsiella* shows green coloration, and *Acinetobacter* spp. appears in colorless or cream colonies. Any carbapenem-resistant isolate was investigated for phenotypic carbapenemase production using the carbapenem inhibition method [39]. For quality control, Klebsiella pneumoniae ATCC 25955 was used.

All isolates were identified to the species level by using MALDI-TOF mass spectrometry. The antibiotic susceptibility pattern was obtained by the Kirby–Bauer disc diffusion method on Mueller–Hinton agar and Vitek-2 according to the EUCAST guidelines.

Patients were considered colonized by any of the organisms studied if one or more of those organisms were isolated from at least one of the swabbed sites. ICU-acquired infection

Table 1: Clinical characteristics of the patients (n= 1971).

	Male	Female
Number of patients	1107	864
Mean age (years)	65.13±14.65	70.15±14.13
Age > 65 years	599	618
Carmeli score	1.54±0.66	1.75±0.77
Previous hospitalization	349	274
Antibiotic treatment	825	648
Surgical patients	53	42
Perioperative infection prophylaxis	13	10
Respiratory diseases	173	140
Cardiovascular diseases	516	437
Chronic renal failure	30	14
Chronic liver diseases	47	27
Cancer	96	70
Hematologic malignancy	3	0
Diabetes mellitus	183	210

was defined if any of the studied pathogen colonies were detected 7 days after ICU admission in previously negative patients.

2.3. Data Analysis. Data were described as mean ± standard deviation (SD), number of cases, or percentages where appropriate. For the statistical analysis of the contingency tables, Chi-square test and Fisher's exact test were used. Statistical significance was considered when $p<0.05$. All statistical calculations were done with GraphPad Prism 7.00.

3. Results

3.1. Demographic Data of Patients. The demographic characteristics and the possible risk factors for infection are summarized in Table 1. A total of 1971 patients, 1107 males and 864 females, were included in this study. The male : female ratio was 1.28 : 1 (56.16% male and 43.83% female). Mean age range was 65.13±14.65 years for males and 70.15±14.13 for females. More than half of the patients were above 65 years of age. One-third of patients (31.6%) had been hospitalized in the previous months, 1473 of 1971 (74.73 %) have been treated with antibiotics, and 44 (2.23%) patients with chronic renal failure came in for regular dialysis.

Carmeli's score was an average of 1.54±0.66 in the male and 1.75±0.77 in female patients. The interpretation of our results shows that the patients included in the study are mostly with probably healthcare-associated or community-acquired infections but with high probability of resistant or multidrug-resistant strains. Among our patients, 96 (4.87%) were surgical patients, from whom 23 (23.95%) received a perioperative infection prophylaxis; therefore the rest of the patients were medical patients and the majority had received at least one group of antibiotics. The main three chronic disease groups presented by patients included in the study were cardiovascular disease, diabetes mellitus, and respiratory diseases regardless of the gender.

3.2. Bacterial Pattern of Colonization upon Admission. Overall, 1971 patients were sampled at one or more sites (nasal, groin, and rectal region) to assess colonization. Bacterial colonization (of 1 or more sites) upon admission was detected in 494 patients (25.06%). Evaluation of spectrum of bacterial colonization on admission shows that there are no significant differences between male and female patients. The results of the cultures from different swabbed sites obtained from the male and female study participants at admission are presented in Table 2.

A number of 687 groin and nasal swabs were collected to detect MRSA; overall positivity has been 5.24%, 4.72% in male and 5.88% in female participants. If we compare the positivity rates between the two sites, groin and nasal cavity, they were 2.91% for the groin and 3.35 % for the nasal. In the same Gram-positive microorganisms, our results show a low rate of VRE colonization, less than 1%, slightly higher in female patients (0.25% versus 0.59%). 162 from 1343 (12.06%) of the studied patients were colonized upon admission with one or more of ESBL-producing organisms. Among the colonizing organisms, 50.76% of the ESBL-producing organisms belonged to the *E. coli* species. There were no significant differences between males (11.96%) and females (12.19%). Among Gram-negative bacteria with a reduced susceptibility to most carbapenem agents (CRE), Enterobacterales were the most prevalent (21.16%). In this order, the principal colonizer organism was *E. coli* (68.07%), followed by *Enterobacter* spp. (11.5%), *Proteus* spp. (7.04%), and *Klebsiella* spp. (4.2%). Nonfermenters were identified as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* and they are almost in the same proportion in males and females, being less than 1% (11 from 1389).

3.3. Bacterial Pattern of Colonization at 7 Days. Patients who were hospitalized for more than 7 days were retested using the same nasal, groin, and rectal swabs for MRSA, VRE, ESBL, and CRE infection (see Table 3).

According to our results, we found that more than 90% of patients were admitted to the ICU without MRSA or VRE infection regardless of gender. Approximately 3 to 4% of patients acquired MRSA infection during the ICU stay, and in male participants 2.99% were found with VRE positivity. We tested the patients during the first day of admission to the ICU and 4% of male and 7.69% of female patients were found to be positive to MRSA upon admittance, and all of them were found to be cleared of infection during the first week of stay. Regarding ESBL and CRE, we found that approximately 88% arrived to the ICU without being colonized, but during their first 7 days of hospitalization nearly 40% got infected with ESBL, while regarding CRE this number is closer to 33%. Upon admittance, 10-15% of patients were positive either to CRE and/or ESBL, and only half of them were found to be clear of infection during their first week of hospitalization.

3.4. Risk Factors for Colonization. According to our analysis, there was no significant association between age, Carmeli's score, and any of the examined comorbidities regarding MRSA or VRE infection upon admittance neither in male

Table 2: Spectrum of bacterial colonization upon admission. MRSA-N: MRSA detected from nasal cavity; MRSA-G: MRSA detected from groin area.

	Male			Female		
	Number of patients [n]	Positivity [n]	Positivity [%]	Number of patients [n]	Positivity [n]	Positivity [%]
MRSA	381	18	4.72%	306	18	5.88%
MRSA-N	381	13	3.41%	306	10	3.26%
MRSA-G	381	9	2.36%	306	11	3.59%
VRE	400	1	0.25%	340	2	0.59%
ESBL	744	89	11.96%	599	73	12.19%
CRE	833	166	19.92%	556	127	22.84%

Table 3: Bacterial colonization of male and female patients upon 7 days of admission. NEG-NEG: no infection was found at admittance and at 7 days. NEG-POS: no infection was found at admittance but the patient was found to be infected at 7 days. POS-NEG: infected at admittance but no infection was found at 7 days. POS-POS: infected at admittance and the patient was found to be infected at 7 days.

	Male				Female			
	MRSA	VRE	ESBL	CRE	MRSA	VRE	ESBL	CRE
NEG-NEG	93%	97.01%	52%	63.11%	88.46%	100%	49.66%	54.86%
NEG-POS	3%	2.99%	33.33%	25.41%	3.85%	0%	37.24%	32.64%
POS-NEG	4%	0%	6%	4.92%	7.69%	0%	6.90%	4.86%
POS-POS	0%	0%	8.67%	6.56%	0%	0%	6.21%	7.67%

Table 4: Analysis of risk factors related to bacterial infections upon ICU admission in male patients. OR: Odds Ratio; ∗: $p<0.05$; ∗∗: $p<0.01$; ∗∗∗: $p<0.001$.

Risk factor	MRSA colonization				VRE colonization			
	Present [n]	Absent [n]	OR	P value	Present [n]	Absent [n]	OR	P value
Age > 65 years	10	208	0.9315	>0.9999	1	219	Infinity	>0.9999
Carmeli's score	10	161	1.568	0.4675	1	183	Infinity	0.46
Respiratory diseases	5	68	1.669	0.3575	0	67	0	>0.9999
Cardiovascular diseases	8	165	0.96	>0.9999	1	193	Infinity	0.485
Chronic renal failure	0	9	0	>0.9999	0	9	0	>0.9999
Chronic liver diseases	0	21	0	0.6123	0	20	0	>0.9999
Cancer	3	30	2.22	0.1972	0	34	0	>0.9999
Hematologic malignancy	0	1	0	>0.9999	0	2	0	>0.9999
Diabetes mellitus	4	53	1.671	0.3257	0	70	0	>0.9999
Risk factor	ESBL colonization				CRE colonization			
	Present [n]	Absent [n]	OR	P value	Present [n]	Absent [n]	OR	P value
Age > 65 years	53	352	1.267	0.3101	87	312	1.334	0.1137
Carmeli's score	46	273	1.497	0.0866	80	246	1.686	0.0039∗∗
Respiratory diseases	15	109	1.015	>0.9999	29	90	1.406	0.168
Cardiovascular diseases	32	312	0.6172	0.0415∗	57	282	0.7463	0.1289
Chronic renal failure	6	10	4.663	0.0074∗∗	7	14	2.117	0.156
Chronic liver diseases	12	20	4.948	0.0001∗∗∗	12	21	2.474	0.022∗
Cancer	9	47	1.455	0.2914	18	51	1.518	0.1538
Hematologic malignancy	1	1	7.432	0.2251	0	2	0	>0.9999
Diabetes mellitus	12	104	0.8257	0.6422	28	90	1.347	0.2105

nor in female patients. In male patients chronic renal failure and liver disease and in female patients only chronic renal failure proved to be significant risk factors for acquiring ESBL infection, with an approximately 5 times higher risk compared to the rest of the participants. Contrarily, cardiovascular diseases in male patients seem to be posing a lower risk of ESBL infection. Regarding CRE, Carmeli's score and chronic liver diseases in male patients, and chronic renal failure in female patients represents a higher risk of infection, while cardiovascular diseases in female participants show a lower risk of colonization (see Tables 4 and 5).

The risk assessment of infection at 7 days showed statistical significance for ESBL and CRE in both male and female patients (see Table 6).

Table 5: Analysis of risk factors related to bacterial infections upon ICU admission in female patients. OR: Odds Ratio; ∗: p<0.05; ∗∗: p<0.01.

Risk factor	MRSA colonization				VRE colonization			
	Present [n]	Absent [n]	OR	P value	Present [n]	Absent [n]	OR	P value
Age > 65 years	13	207	1.017	>0.9999	2	241	Infinity	>0.9999
Carmeli score	9	166	0.7349	0.6254	2	189	Infinity	0.5062
Respiratory diseases	6	53	2.217	0.128	1	66	4.121	0.3558
Cardiovascular diseases	11	159	1.275	0.8077	1	176	0.9205	>0.9999
Chronic renal failure	0	4	0	>0.9999	0	5	0	>0.9999
Chronic liver diseases	1	7	2.361	0.3879	0	12	0	>0.9999
Cancer	2	24	1.375	0.6571	1	32	9.563	0.185
Hematologic malignancy	-	-	-	-	-	-	-	-
Diabetes mellitus	3	68	0.6471	0.7734	0	82	0	>0.9999
Risk factor	ESBL colonization				CRE colonization			
	Present [n]	Absent [n]	OR	P value	Present [n]	Absent [n]	OR	P value
Age > 65 years	50	376	0.8673	0.5843	83	311	0.8217	0.4234
Carmeli score	44	288	1.254	0.3828	64	239	0.8879	0.6051
Respiratory diseases	9	81	0.7726	0.6011	28	74	1.44	0.1472
Cardiovascular diseases	35	277	0.8279	0.4564	49	238	0.5433	0.0038∗∗
Chronic renal failure	4	6	5.024	0.0241∗	4	4	3.624	0.0754
Chronic liver diseases	3	15	1.46	0.472	6	12	1.809	0.2501
Cancer	8	37	1.627	0.2362	10	38	0.9246	>0.9999
Hematologic malignancy	-	-	-	-	-	-	-	-
Diabetes mellitus	19	118	1.217	0.5518	28	108	0.8921	0.7208

Table 6: Analysis of risk factors related to bacterial infections after 7 days. OR: Odds Ratio; ∗: p<0.05; ∗∗∗: p<0.001.

	MRSA colonization				VRE colonization			
	Present [n]	Absent [n]	OR	P value	Present [n]	Absent [n]	OR	P value
Male	3	93	0.6505	0.7801	2	65	11.94	0.0553
Female	4	92	0.6957	0.6163	0	181	0	0.5457
	ESBL colonization				CRE colonization			
	Present [n]	Absent [n]	OR	P value	Present [n]	Absent [n]	OR	P value
Male	50	78	4.79	<0.0001∗∗∗	31	77	1.665	0.031∗
Female	54	72	5.404	<0.0001∗∗∗	47	79	2.104	0.0006∗∗∗

4. Discussion

In this study, we have analysed the incidence and pattern of bacterial colonization including MDR Gram-positive (MRSA, VRE) and Gram-negative (ESBL, CRE) germs in ICU patients upon admission and after 7 days. Multidrug resistance in Enterobacterales and nonfermenting Gram-negative bacilli represent a real challenge to the hospitals or healthcare institutions. Carriage or infections with these isolates can result in compromised treatment options and high mortality rates in patients. Therefore, early detection of infected or colonized patients is compulsory for the best patient management and to prevent the patient-to-patient transmission or environmental contamination. In addition, identifying the risk factors associated with MDR bacterial infections and the most commonly occurring MDR strains may be of use to hospital antimicrobial drug management and the prevention of nosocomial contamination [40]. Most investigations of risk factors for multidrug-resistant strains have been hospital-based and focused on them. The risk factors can be exposure to antibiotics and multidrug-resistant strains in the environment (recent antibiotics and/or hospitalization, history of multidrug-resistant strains, colonization pressure, comorbidity, and dialysis) and special conditions that facilitate colonization and infection (illness severity, wounds, indwelling devices, etc.). Some risk factors have more importance than others, but they can be evaluated and updated. The prevalence and patterns of resistance vary significantly by geographical area, location, size, and facility type, with each institution having a unique pattern. Some hospitals screen all admissions for multidrug-resistant strains colonization. Moreover, an important recommendation is to follow the guidelines regarding the prevention of nosocomial infections. Our institution recently introduced Carmeli's score for evaluation of risk factors for infections with resistant or multidrug-resistant bacteria and it allows doing a prediction. A better diagnostic information includes culture of the causative pathogen and the screening for them

was performed only for one year (2017). Rising rates of Gram-negative multidrug-resistant bacteria have been documented. Therefore, it is essential to collect these data and not to assume that our ICU issues are the same as those reported by others.

The overall MRSA carriage rate of 5.2% found in our study is less than 22.5%, which was reported by Ray et al. [41], and greater than 3%, as reported by other scientists [42, 43]. The slightly higher prevalence of MRSA carriage found in the present study could be explained by the relatively small number of patients examined, and the significantly higher rate of infection in Ray's report could be due to the multiple sites of sampling which could detect the presence of MRSA with more accuracy. One of the most prevalent sites of MRSA has been reported to be the nose and our data is in agreement with that observation [41]. The incidence of MRSA infection in the ICU was nearly the same as that on admission in the case of male patients, but females were more likely to be affected on admission than being infected in the hospital. We observed that the rate of MRSA eradication during the hospitalization was 100%, which can be attributed to the absence of correlation of the MRSA carriage with different risk factors and to the correctly chosen and applied antibiotic therapy. Previous studies have identified various risk factors for VRE colonization including advanced age, central venous catheterization, extended hospitalization, haematologic malignancies, haemodialysis, exposure to multiple antibiotics, and prolonged duration of antibiotic therapy [44]. Karki et al. in a study detected 17.5% prevalence of VRE colonization on the day of screening [45]. Contrarily, our results show a significantly low rate of VRE colonization upon admission (0.4%), which is slightly higher in female patients. Seven days of ICU stay proved no higher risk of VRE infection regardless of gender. The beta-lactam antimicrobial agents are among the most commonly used classes of antibiotics. Consequently, resistance to β-lactams by production of β-lactamases is the most common cause of resistance to these drugs. Majority of the ESBLs are found in *Klebsiella* spp. and *Escherichia coli* [46]. Our study results identified 12.06% prevalence rate for ESBL producers and it correlates well with data from an Iranian report but a Canadian research group found less occurrence in their patient screening study [47, 48]. Regarding ICU stay, we concluded that at least 7 days of admittance poses an approximately five times higher risk of ESBL contamination. According to our results, chronic renal failure and liver diseases might contribute to such high prevalence; however, we also found that more than half of the already ESBL-infected patients staying in the ICU were still carrying the MDR strain after one week of hospital care. As a subclass of beta-lactam antibiotics, the carbapenems are usually applied as spare drugs in the treatment of severe bacterial infections that are resistant to the commonly used antibiotics. The current study showed very high incidence (21.06%) of CRE infection and more in the female than male patients at admission and after 7 days. Upon admittance, more than 10% arrived already carrying the carbapenemase expressing strain, and half of these colonizers were positive to CRE after 7 days. They might have contributed to that during the hospitalization; more than 30% of the CRE-negative patients acquired CRE infection. Apart from hospitalization, chronic liver diseases and Carmeli's score proved to be a statistically significant risk factor in men, while in the case of women we found a tendency that points to the possible connection of chronic renal failure to CRE infection.

According to our current results, we can report that nosocomial infections can occur in the Intensive Care Unit of Emergency Clinical County Hospital of Oradea. The most common isolates were ESBL and CRE strains, but in comparison Gram-positive MDR bacteria such as MRSA and VRE were detected in a significantly less proportion. It is promising that, in addition to the lower level of colonization, MRSA and VRE were showing a better response to antimicrobial therapy compared to GN MDR bacteria. However, ESBL and CRE pose a difficult problem by either medical or economic reasons. The number of nosocomial GN infections was remarkably high, which can be attributed to the fact that more than half of the originally infected patients remain carriers even after being a week after admittance into intensive care, and it should also be noted that the hospital environment contributes to the selection of strains displaying one or more resistance mechanisms against the antibiotics; therefore we have to prepare for the appearance of more persistent bacteria in the future. We must underline the importance of rational antibiotic strategies, the appropriate personal hygiene and preventive methods, and the consideration of auxiliary medical therapies, such as the application of natural compounds that might offer favourable alternatives based on their antioxidant and disinfectant properties [49, 50]. Finally, the risk factor assessment should be done before selecting empiric antibiotic therapy and it could improve the prognosis of some patients and help the development of infection control policies and procedures, as well as the review of antibiotic utilization and its relationship to local antibiotic resistance patterns, together with the development of guidelines for the rational use of antimicrobial therapy.

5. Conclusion

Our study is in accordance with other findings and supports the importance of identifying and managing risk factors involved in the mechanism of colonization of the human patients with potentially multidrug-resistant pathogenic bacteria during hospitalization, especially in the intensive care unit.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Dana Carmen Zaha and Rita Kiss contributed equally to this work.

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Research Article

Hepatitis B Birth Dose Vaccination among Vietnamese Children: Implications for the Expanded Program on Immunization

Hao Nguyen Si Anh,[1] **Hoang-Long Vo**,[1] **Long Hoang Bao**,[2] **Hien Tran Minh**,[1] **Ha Tran Thi Thu**,[1] **and Vu Duy Kien**[3]

[1]*Institute for Preventive Medicine and Public Health, Hanoi Medical University, Hanoi, Vietnam*
[2]*Institute of Gastroenterology and Hepatology, Hanoi, Vietnam*
[3]*Oncare Medical Technology Company Limited, Hanoi, Vietnam*

Correspondence should be addressed to Hao Nguyen Si Anh; anhhao5896@gmail.com and Hoang-Long Vo; vohoanglonghmu@gmail.com

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Background. This study assesses the prevalence of Vietnamese children receiving the hepatitis B (HepB) vaccine birth dose and explores its associated socioeconomic factors. *Methods.* We used the data of the Multiple Indicator Cluster Survey, 2014. We estimated the overall percentage of HepB birth dose vaccination among 0–23-month-old children and its percentages according to selected characteristics. Multiple logistic regression was applied. *Results.* 62.8% of children received the HepB vaccine birth dose. The prevalence rates by selected factors ranged from 35.3% to 76.7%. The categories with the lowest prevalence rates were children who had low birth weight (41.6%), had a mother aged less than 20 years (35.3%), had a mother with primary or less education (42.7%), belonged to ethnic minorities (30.3%), resided in rural areas (59.9%), and were in the 1st quintile of mother's socioeconomic status (38.6%). Receiving HepB vaccine birth dose was associated with child's birth weight, mother's age, mother's education, socioeconomic status, and ethnicity. *Conclusions.* This study identified vulnerable groups, upon which policy-makers should focus their efforts to equitably and sustainably tackle birth dose HepB vaccine coverage as well as the full vaccination coverage, thereby promoting long-lasting herd immunity in this country.

1. Introduction

Hepatitis B (HepB) is an infection of the liver caused by the HepB virus that attacks liver cells [1]. The HepB virus is transmitted by exposure to blood or other bodily fluid of an infected person. HepB results in more than 780,000 deaths every year, mostly from liver cancer and cirrhosis, while an estimated 257 million people are living with chronic HepB [2]. HepB is considered as a major global health problem, especially in the developing countries such as Vietnam [3, 4].

The Western Pacific region is well-known as an area with a very high burden of HepB, accounting for nearly half of all chronic HepB cases worldwide [3, 4]. Vietnam, with about 8.6 million people who are carriers of virus, is one of 11 countries in the Western Pacific region with the highest prevalence of HepB [5, 6]. Previous studies in different regions in Vietnam showed that the incidence of HepB has been estimated approximately 10%-20% of the population, in which the viruses are mainly vertically transmitted from mother to child [7–9]. Most people currently living with HepB virus infection were born before HepB vaccine was widely available and used in the infancy [2].

The HepB birth dose is a monovalent vaccine containing surface proteins of the HepB virus absorbed by aluminum hydroxide or monophosphoryl lipid A adjuvant [10]. Surface antigens extracted from HBsAg-positive human plasma are asymptomatic or recombinant in yeast cells [10, 11]. In 2006, following the World Health Organization (WHO) recommendation, Vietnam officially implemented the first dose of HepB vaccination for the infants within the first 24 hours after birth [12]. A study conducted in 51 provinces across Vietnam revealed that the rate of positive children with HBsAg in the

period of 2008-2011 fell to less than 2% compared to 3.62% during 2000-2003 [5, 13]. The coverage of HepB vaccination within first 24 hours after birth also reached 76.6% at the end of 2017 after 3 decades of the Vietnamese government's efforts [6].

In Vietnam, the Kinh is the main ethnic group, and other ethnic groups account for up to 14% of the national population [14]. While Vietnam is putting more and more effort to maintain the coverage of HepB birth dose, the country continues to face the problem of "vaccine hesitancy", one of the global health threats [15]. More than 8.8% of Vietnamese women and 12.3% of Vietnamese men carry viruses for coming decades [1, 5]. These are considered as great challenges that require enduring and comprehensive efforts for Vietnam to achieve the goal of "HBsAg prevalence of less than 0.1% in 5-year-old children" for the period 2018-2030 control [6].

Recognizing the neglected aspects of the HepB birth dose coverage across the country may contribute to developing policy to expand the prevention interventions for mother-to-child transmission of HepB virus. Exploring key socioeconomic factors that are as the barriers to the HepB birth dose vaccination could pave pathway for scaling up other specific healthcare interventions and therefore could contribute to investigation, prevention, and management of healthcare-associated HepB infections among both mothers and newborns in the community. In the present paper, we used the most updated data from the Multiple Indicators Cluster Surveys (MICS) of the United Nations International Children's Emergency Fund (UNICEF) in the survey round of 2014 to assess the prevalence of Vietnamese children who received the birth dose of HepB vaccination and to identify socioeconomic factors associated with receipt of the birth dose.

2. Methods

2.1. Data Source. Data from the MICS 2014 was used for this study. The MICS was conducted by the General Statistics Office in collaboration with the Ministry of Health and the Ministry of Labor, Invalids and Social Affairs. Financial and technical supports for the survey were provided by the United Nations Children's Fund and the United Nations Population Fund. The MICS is nationally representative survey covering a broad range of issues affecting the health, development, and living conditions of Vietnamese women and children. The number of children aged 0-23 months included in the 2014 MICS was 1382 [16].

2.2. Variables and Indicators. The main outcome variable in our study was a binary variable specifying whether a 0–23-month-old child received the first dose of HepB vaccination within 24 hours after birth. Data for the available were extracted retrospectively from the MICS 2014. In these surveys, data regarding the HepB birth dose vaccination were obtained from vaccination cards. If no vaccination card was available, the interviewers would ask mothers whether their child received a vaccination against HepB first dose vaccination [12, 16].

The explanatory variables are as follows: child's sex (male/female), low birth weight defined as less than 2,500 grams (yes/no), mother's age (<20/20-35/36-49), mother's education (primary or less/lower secondary/upper secondary and higher), ethnic group (Kinh/Hoa ethnicity and minority ethnic group), living area (rural/urban), and mother's socioeconomic status.

Mother's socioeconomic status (known as household's socioeconomic status) was measured as an asset-based wealth index and was constructed using principal component analysis (PCA). The MICS dataset included the Household Wealth Index which was calculated by the GSO of Vietnam. The index was based on the ownership of consumer goods, dwelling characteristics, water and sanitation, and other characteristics related to household wealth. Weights (factor scores) were assigned to correspond with individual household assets [16]. The details of the method used for estimating the wealth asset index are described elsewhere [16]. Five categories (quintiles) were ranged from the poorest to the richest.

2.3. Data Analysis. Both descriptive and analytical methods were used in the present paper. We estimated the overall percentage of HepB birth dose vaccination among 0–23-month-old children and the percentage according to sex, region, area, ethnicity, mother's education, and socioeconomic status. The Wald's Chi-square test was applied to compare the differences of receiving the HepB dose vaccination within 24 hours after birth among groups. The multiple logistic regression was used for the binary primary outcome variable to explore the factors associated with HepB birth dose vaccination. The cumulative probability of being vaccinated among ethnicities at age t among ethnic groups was estimated by the inverse Kaplan–Meier survival function (or 1–SKM(t)), also known to measure the fraction of children receiving the birth dose HepB vaccination for a certain amount of time [17]. All statistical analyses were carried out using Stata® 13.1 (StataCorp LLC, USA), with weighting factors for children from the dataset. A significance level of p-value <0.05 was used.

2.4. Research Ethics. This study was conducted on secondary data from the MICS with all identifiable information removed. The survey had obtained informed consent from the mothers before administering survey questionnaires and the consenting process had stated that data can be analyzed in subsequent analyses without retaking informed consent. All information in the original dataset was collected confidentially.

3. Results

3.1. Prevalence of Receiving the Hepatitis B Birth Dose Vaccination. Prevalence rates and 95% confidence intervals for receiving the birth dose of HepB vaccination in each category by the selected socioeconomic factors are presented in Table 1. In 2014, 62.8% of children received the birth dose of HepB vaccination. The prevalence rates by selected factors ranged from 35.3% to 76.7%. The categories with the

Table 1: Prevalence of receiving timely the birth dose of hepatitis B vaccination by selected socioeconomic factors among Vietnamese children aged 0–23 months, MICS 2014 (n=1382).

Characteristics	Un-weighted sample size n (weighted %)	Weighted prevalence % (95%CI)	Chi-Square p-value
Sex			0.7
Male	741(54)	62.3(57.6,66.7)	
Female	641(46)	63.4(58.8,67.7)	
Low birth weight			<0.01
No	1311(95.4)	63.8(60.2,67.3)	
Yes	71(4.6)	41.6(29.2,55.2)	
Mother's age			<0.01
<20	91(5.5)	35.3(25.0,47.3)	
20-35	1131(83.4)	64.6(60.8,68.2)	
36-49	160(11.1)	62.7(53.8,70.8)	
Mother's education			<0.01
Primary or less	259(16.1)	42.7(34.0,51.8)	
Lower secondary	479(36.4)	63.6(58.6,68.4)	
Upper secondary and tertiary	644(47.5)	68.9(64.5,73.1)	
Ethnicity			<0.01
Kinh	1062(83.7)	69.1(65.6,72.4)	
Minority	320(16.3)	30.3(23.7,37.9)	
Area			<0.01
Urban	526(29.8)	69.6(64.5,74.3)	
Rural	856(70.2)	59.9(55.2,64.4)	
Mother's wealth status			<0.01
1st quintile (poorest)	329(19.3)	38.6(31.3,46.4)	
2nd quintile	245(19.6)	66.2(59.2,72.6)	
3rd quintile	250(20.1)	62.2(55.5,68.5)	
4th quintile	284(21.5)	76.7(69.8,82.4)	
5th quintile (richest)	274(19.5)	68.5(62.1,74.3)	
Overall	1382(100)	62.8(59.2,66.3)	

CI: confidence interval.

lowest prevalence rates were children having low birth weight (41.6%), had a mother aged less than 20 years (35.3%), had a mother with primary or less education (42.7%), belonged to ethnic minorities (30.3%), resided in rural areas (59.9%), and were in the 1st quintile mother's wealth status (38.6%). The difference in prevalence rates among all categories of each factor were significant (p-value <0.05) for most selected socioeconomic factors (except for the children gender).

The two curves of cumulative proportion for children receiving the birth dose of HepB vaccination belonging to Kinh/Hoa ethnicity and belonging to minority by children age in days are shown in Figure 1. For Kinh/Hoa children, on birth day the birth dose vaccination rate started at 69.3%, then the birth dose rate rose sharply to above 90% at age 120 days and the following days (red curve in Figure 1). For minority, at age 1 day, more than 35% children received the birth dose, then the proportion of receiving the birth dose increased to 80% at age 145 days and following days (blue curve in Figure 1). However, the wide gap of receiving HepB vaccination between Kinh/Hoa and minority was still significant (Figure 1).

3.2. Socioeconomic Factors Associated with HepB Birth Dose Vaccination. Compared with the prevalence of receiving the birth dose of HepB vaccination among children with low birth weight, the odds was twice higher for the children with normal birth weight (OR 2.13; 95%CI 1.16-3.89). Kinh/Hoa children had significantly higher odds of HepB birth dose vaccination than individuals from ethnic minorities (OR 3.15, 95%CI: 2.04–4.88). Mother's age was significantly associated with increased prevalence of receiving the HepB birth dose vaccination for their children. Children whose mothers had higher education were significantly more likely to have had completion of the HepB birth dose vaccination compared with those had mothers experiencing primary or less education. The odds of HepB birth dose vaccination were higher in children who belonged to the families with better economic status (Table 2).

4. Discussion

In a Kate Whitford et al.'s report (2018), most immunization schedule in all studies included a first dose of HepB vaccine

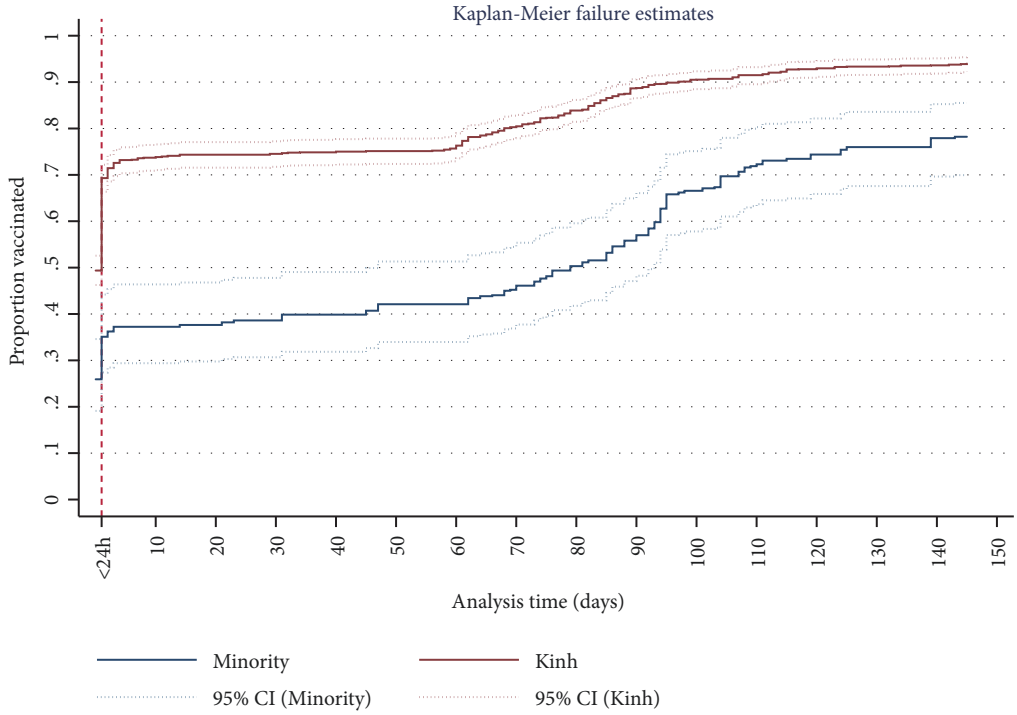

Figure 1: Cumulative proportions of receiving the birth dose of HepB vaccination by age in days among Vietnamese children belonging to Kinh/Hoa ethnicity and minority ethnic groups.

within 24 hours after birth except for one from Italy [18], where the birth dose was only included in the targeted vaccination schedule [19]. In Vietnam, in the decision number 2620/QD-BYT guiding the implementation of birth dose HepB vaccination in 2012, the Ministry of Health set the goal that the immunization coverage of HepB birth dose should reach at least 65% [20]. In our study, the prevalence of receiving HepB vaccine birth dose within 24 hours (62.8%) did not reach the target. However, it reported a consistently increasing trend since the HepB vaccine dose was recommended to be given within 24 hours after birth in 2006. The WHO has reported that the global HepB immunization coverage with three doses during infancy reached 84% in 2015 [21]; nevertheless, the global birth dose coverage of HepB vaccine remained low, at an estimated 39% in 2015 [22]. So, the present proportion of timely HepB birth dose vaccination among Vietnamese children was overall acceptable. This finding was important for the Vietnam's national immunization program to recognize the existing gap which might result in the vaccination proportion not reaching the goal set by the Ministry of Health, thereby continuously improving the immunization service in highly endemic countries like Vietnam, where 10.5% of pregnant women are HepB virus carriers [23] as well as where a high proportion of HepB virus infections are acquired perinatally [24, 25].

In our study, low birth weight children had significantly lower vaccination rates for HepB within 24 hours after birth. Saari et al. (2003) reported that vaccination rates could be affected by the infant's birth weight [26]. Infants with a birth weight of below 2500 grams may have weaker immune response; therefore, the HepB vaccine first dose cannot be requested by their doctors [27].

In our study, the prevalence of receiving HepB vaccine dose within 24 hours after birth was significantly higher in urban areas than in rural areas. Previous studies in Vietnam and elsewhere also found that urban areas had higher coverage of full immunization [28, 29]. This may be explained by higher availability and better quality of vaccination services in urban areas through concentrated efforts by the organizations and individuals dealing with vaccination service. Other explanations may be difficult logistically, particularly for home deliveries as cold-chain infrastructure is limited in remote rural areas. There are two types of vaccinations known as expanded vaccination (free-of-charge) and service vaccination (parents have to pay for vaccination) in Vietnam. Public health facilities having their assigned functions and tasks of vaccination for all children in the expanded immunization program (EPI) are compulsorily available throughout this country. Nevertheless, private immunization service centers, which are also allowed to register with the local Department of Health to implement vaccination in the EPI, are opened more in urban areas where the citizens have better socioeconomic status. To our knowledge, in rural areas known to have lower socioeconomic status, the uptake of HepB birth dose vaccination at most commune health centers and general hospitals may be due to the free-of-charge vaccination.

In Vietnam, there is a fact that women under the age of 20, who are in attending-school age, are not able to get married

Table 2: Selected factors associated with receiving timely the birth dose of hepatitis B vaccination among children aged 0-23 months, 2014: multivariate logistic regression analysis (n=1382).

Characteristics	OR (95% CI)
Sex	
Male	1
Female	1.03(0.79,1.34)
Low birth weight	
Yes	
No	2.13∗(1.16,3.89)
Ethnicity	
Minority	1
Kinh/Hoa	3.15∗ ∗ ∗(2.04,4.88)
Area	
Rural	1
Urban	1.25(0.86,1.82)
Mother's age	
<20	1
20-35	2.24∗∗(1.26,3.98)
36-49	2.17∗(1.1,4.31)
Mother's education	
Primary or less	1
Lower secondary	1.68∗(1.11,2.53)
Upper secondary and tertiary	1.74∗(1.13,2.67)
Mother's wealth status	
1st quintile (poorest)	1
2nd quintile	1.65∗(1.06,2.56)
3rd quintile	1.18(0.73,1.9)
4th quintile	2.06∗(1.17,3.64)
5th quintile (richest)	1.17(0.65,2.11)

OR: Odd ratio; CI: confidence interval
*,**,***: significant at 0.05, 0.01, and 0.001, respectively.

and give birth later. On the other hand, a finding of Linh Cu Le et al. reported that a risk of unintended pregnancy was 1.5 times higher in Vietnamese women marrying before 20 than those later or early marriage associated with unintended pregnancy [30]. Therefore, their awareness of raising a child can be incomplete. Perhaps the reasons above are appropriate to explain one of the present findings that children having mothers less than 20 years old were less likely to receive HepB vaccine birth dose compared with those having mothers aged over 20 years. The gap between the rich and the poor, between Kinh and other ethnic minority groups, between low educational level and high one, and between ages is concerning issues in Vietnam [14, 31]. Also in this study, we found that mother's education, household wealth, and mother's ethnicity were associated with timely immunization completion for HepB vaccine birth dose in the multivariate analysis. In particular, children of Kinh/Hoa ethnicity were more likely to receive timely immunization completion for HepB vaccine birth dose compared with children from minority ethnic groups. This difference might be due to the fact that people of different ethnicity had different attitudes, health-seeking behaviors, and socioeconomic status. In Vietnam, the Kinh who live mainly in the plains, near the rivers, and in urban areas are more likely to benefit from better socioeconomic conditions [32]. On the other hand, most ethnic minority groups, who generally have relatively poorer socioeconomic conditions and lower literacy rate, are living in the highlands and rural and mountainous areas [32]. Hence, women from ethnic minority groups have difficulties in approaching in expanded immunization services like the HepB birth dose vaccination. We found that although the proportion of receiving HepB vaccine birth dose for both Kinh/Hoa ethnicity and ethnic minority groups gradually increased at following days, these figures for timely vaccination within 24 hours as recommended were low and in particular for HepB birth dose vaccination rate of ethnic minority groups were very low, at estimated below 40%. According to issued document in the 9th National Congress, equity among all ethnic groups was recognized in The Vietnamese Constitution as a priority [14]. This finding is important for this country's government as well as policy-marker to understand the barriers to preventing mothers' access to HepB birth dose vaccination. The present finding suggests that narrowing the gap between the Kinh/Hoa ethnicity and minority ethnic group is still a long-lasting way in the development and implementation of a strategy to improve timely birth dose coverage for

HepB vaccine, in particular for a low-middle-income country like Vietnam. The poverty gap among ethnic groups has been documented in a previous report by Hai-Anh Dang, suggesting that the vaccination rates for Vietnamese children need to increase among ethnic minority women, considered as the short-term approach [33]. Therefore, our finding provides important evidence for the scientists and policy-makers in advancing the accessibility for HepB vaccine first dose within 24 hours after birth, which contributes to the full immunization coverage in the EPI.

The findings from this study have meaningful policy implications. This is the first study that assessed the coverage of timely HepB vaccine birth dose according to socioeconomic factors using the MICS data, which are the national representative immunization survey data in Vietnam. Importantly, the resource mobilization for the immunization in Vietnam is currently limited, not meeting the demand of expanded immunization [34]. In the context of Vietnam being a middle-income country, international funding support for expanded immunization is declining [34]. Hence, Vietnam has faced the challenge to meet the huge demand for the investment resources for expanded vaccination, including the consolidation and supplementation of cold-chain equipment for the vaccine preservation and the training of qualified health workers in the immunization [34]. This study identified vulnerable population groups (children with low birth weight, living in rural areas, mothers aged less than 20 years, mothers with low education, mothers from ethnic minorities, and poor socioeconomic status), upon which the policy-makers should focus their efforts to equitably and sustainably tackle the inequalities in the receipt of HepB birth dose as well as the full vaccination coverage, thereby promoting long-lasting herd immunity in Vietnam. Also based on the important findings in the present study, the public health decision-makers can understand that identified vulnerable populations above are underserved, which helps them consider integrating vulnerable populations-related issues of vaccination into existing national programmes for newborn HepB vaccination at four administrative levels of health establishments (central level, provincial level, district level, and commune), as well as building the evidence-based guidance of priority actions for the vaccination. Country policy-makers and immunization program implementers should consider the fact that the limited resources towards the Vietnam EPI need allocating most effectively in remote mountainous areas where the main habitats of disadvantaged populations exist.

However, we acknowledge some limitations to this study. First, due to the limitation of the cross-sectional study design of the MICS, the results should be interpreted with caution so that they are not interpreted as implying causality. Second, the estimations of receiving HepB vaccine birth dose were derived from available information on vaccination cards and/or reported by mothers. Therefore, some children may have been vaccinated but did not have immunization card and were excluded from the study. In addition, mothers may have forgotten to report the vaccination of their children during their interviews. These would underestimate the birth dose of HepB vaccination coverage in this study. Finally, the cultural aspects related to uptake of HepB birth dose vaccination could not be assessed, such as acceptability and attitudes of Vietnamese women towards the vaccine quality.

5. Conclusions

We found that the prevalence of receiving the first dose of HepB vaccine within 24 hours after birth did not meet the target of Vietnam (the immunization coverage of HepB birth dose should reach at least 65%). It is important to continue to coordinate with the WHO to support the implementation of newborn HepB vaccination; nevertheless, the targeted interventions in vulnerable population groups including child's low birth weight, mother's age less than 20, mother's low education, mother's low socioeconomic status, and child's ethnicity should be prioritized. There was a significant gap in the HepB vaccine birth dose coverage between the Kinh/Hoa ethnicity and minority ethnic groups, suggesting a need to improve both access and demand for HepB vaccine after birth among the other ethnic groups, in particular for minority ethnic groups living in remote and poor conditions. Furthermore, the policy developments in the HepB birth dose vaccination in particular and recommended vaccinations of the EPI in general should be established based on vulnerable populations, which leads to the sustainable interventions to decrease risk for healthcare-associated infections.

Data Availability

The MICS datasets existing are open to public; all users were allowed to free access after requesting to use. The raw data of the 2014 survey round of MICS was obtained with the approval to use the data for this study. UNICEF MICS encourages all users to share the research findings. The details of the MICS dataset source are described in UNICEF website (http://mics.unicef.org/).

Disclosure

The views expressed in this article are solely those of the authors and do not represent the official positions of the organizations the authors affiliated with.

Conflicts of Interest

No potential conflicts of interest were reported by the authors.

Authors' Contributions

Hao Nguyen Si Anh, Hoang-Long Vo, and Vu Duy Kien designed and conceptualized the study. Hao Nguyen Si Anh analyzed the data. Hao Nguyen Si Anh, Hoang-Long Vo, Hien Tran Minh, and Ha Tran Thi Thu interpreted the results and drafted the manuscript. Vu Duy Kien and Long Hoang Bao commented and provided the important knowledge for completing the final manuscript. Long Hoang Bao edited English for the final manuscript. All authors read and approved the final manuscript.

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Research Article

Impact of Healthcare-Associated Infections on Length of Stay: A Study in 68 Hospitals in China

Huixue Jia,[1] Liuyi Li,[1] Weiguang Li,[2] Tieying Hou,[3] Hongqiu Ma,[4] Yun Yang,[5] Anhua Wu,[6] Yunxi Liu,[7] Jianguo Wen,[8] Huai Yang,[9] Xiaoli Luo,[10] Yawei Xing,[11] Weihong Zhang,[12] Yinghong Wu,[13] Lili Ding,[14] Weiping Liu,[15] Ling Lin,[16] Ying Li,[1] and Meilian Chen[13]

[1] *Peking University First Hospital, Beijing 100034, China*
[2] *Shandong Provincial Hospital, Jinan 250021, China*
[3] *Guangdong General Hospital, Guangzhou 510008, China*
[4] *The First Affiliated Hospital of Anhui Medical University, Hefei 230022, China*
[5] *The First Hospital, Shanxi Medical University, Taiyuan 030001, China*
[6] *Xiangya Hospital, Central South University, Changsha 410008, China*
[7] *General Hospital of PLA, Beijing 100853, China*
[8] *The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China*
[9] *Guizhou Provincial People's Hospital, Guiyang 550002, China*
[10] *Jiangxi Provincial Children's Hospital, Nanchang 330006, China*
[11] *Fourth Hospital of Hebei Medical University, Shijiazhuang 050019, China*
[12] *Jiangsu Province Hospital, Nanjing 210029, China*
[13] *Peking University People's Hospital, Beijing 100044, China*
[14] *The First Affiliated Hospital of Xinjiang Medical University, Urumqi 830054, China*
[15] *Inner Mongolia People's Hospital, Hohhot 010017, China*
[16] *Heilongjiang Provincial Center for Disease Control and Prevention, Harbin 150030, China*

Correspondence should be addressed to Liuyi Li; lucyliuyi@263.net

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Healthcare-associated infections (HAIs) not only bring additional medical cost to the patients but also prolong the length of stay (LOS). 2119 HAI case-patients and 2119 matched control-patients were identified in 68 hospitals in 14 primary sampling provinces of 7 major regions of China. The HAI caused an increase in stay of 10.4 days. The LOS due to HAI increased from 9.7 to 10.9 days in different levels of hospitals. There was no statistically significant difference in the increased LOS between different hospital levels. The increased LOS due to HAI in different regions was 8.2 to 12.6 days. Comparing between regions, we found that the increased LOS due to HAI in South China is longer than other regions except the Northeast. The gastrointestinal infection (GI) caused the shortest extra LOS of 6.7 days while the BSI caused the longest extra LOS of 12.8 days. The increased LOS for GI was significantly shorter than that of other sites. Among 2119 case-patients, the non-multidrug-resistant pathogens were detected in 365 cases. The average increased LOS due to these bacterial infections was 12.2 days. *E. coli* infection caused significantly shorter LOS. The studied MDROs, namely, MRSA, VRE, ESBLs-*E. coli*, ESBLs-KP, CR-*E. coli*, CR-KP, CR-AB, and CR-PA were detected in 381 cases (18.0%). The average increased LOS due to these MDRO infections was 14 days. Comparing between different MDRO infections, we found that the increased LOS due to HAI caused by CR-PA (26.5 days) is longer than other MDRO infections (shorter than 19.8 days).

1. Introduction

Healthcare-associated infections (HAIs) not only threaten the patients' health and life but also bring additional economic burden to the patients and healthcare system including direct economic loss and prolonged hospitalization. Total hospital length of stay (LOS) is known to be prolonged by the occurrence of HAI.

An increased length of stay of 5 days due to HAIs in the ICU was estimated in a study of France [1]. The excess of days of hospitalization for infected patients in ICU was 7.7 days in another study [2].

For different infection sites, the extra LOS was 27.1 days, 22.2 days, and 19.2 days for CLABSI, VAP, and CAUTI, respectively, in adult and pediatric ICUs [3]. The mean LOS attributable to CLABSIs was 19 days in another study [4]. The extra length of stay was 3.48 days for BSI, 3.59 days for UTI, 7.23 days for SSI, and 11.52 days for VAP in medical-surgical ICU [2].

Most studies show that multidrug-resistant organism (MDRO) infections cause extra LOS of 2.0–12.7 days compared with those caused by susceptible strains [5–11].

However, many studies on the increased LOS due to HAIs had poor homogeneity and comparability, because most studies were limited to infections caused by a single site or a single organisms, and the characteristics of patients in the studies were different [12–14].

At present, many studies have reported the effect of HAIs on the LOS, but the LOS varies according to the site of infection, infection of pathogens, and different hospital levels. Previous studies have not systematically analyzed the above related factors, and there is a lack of large scale and large sample research.

Our study aimed to evaluate the impact of HAIs on LOS from different hospital levels, different regions, different infection sites, different pathogens, and different MDROs systematically in China.

2. Materials and Methods

2.1. Sampling Methods. This survey was conducted in 68 hospitals in 14 primary sampling provinces (Shandong, Guangdong, Anhui, Shanxi, Hunan, Henan, Guizhou, Jiangxi, Hebei, Jiangsu, Beijing, Xinjiang, Inner Mongolia, and Heilongjiang) of 7 major regions of China (Northeast, North, Central, East, South, Northwest, and Southwest). Each province had at least one provincial or ministerial level general hospital, one prefectural or municipal level general hospital, and/or one district or county level general hospital.

2.2. Patients. From January 1 to December 31, 2015, 50 patients with HAIs were randomly selected in one hospital including 10 lower respiratory tract infections [(LRTI) including ventilator-associated pneumonia (VAP)], 10 urinary tract infections [(UTI) including catheter associated urinary tract infections (CAUTI)], 10 gastrointestinal infections [(GI) including infectious diarrhea, gastrointestinal infection, and antimicrobial associated diarrhea], 10 surgical site infections (SSI), and 10 blood stream infections [(BSI) including central catheter associated blood stream infection (CLABSI)]. If the infections in one site were less than 10 cases, all cases were investigated. One control patient was selected for one case-patient. The matching principle included the same sex, age difference of less than 5 years, the same or similar first diagnosis (the main diseases in the hospital), and the same or similar surgical procedure if it applies. The control-patients should have stayed in the hospital more than 48 hours and have no incurred healthcare-associated infections. If the HAI subject is 2 to 5 years old with the age difference being less than 1 year or if the HAI subject is less than 2 years old, then the matching age is the same. The case-patients were excluded if they had the following conditions: (1) patients with 2 or more HAIs; (2) patients in geriatric ward or intensive care unit or with long-term coma (> 1 month), long stay in hospital (> 3 months) due to vegetative or other noninfectious causes (such as medical dispute); (3) patients with infection upon admission; or (4) patients with no matched controls.

2.3. Pathogens. This study mainly monitored multidrug-resistant or non-multidrug-resistant *Staphylococcus aureus* (SA), *Staphylococcus epidermidis* (SE), *Enterococcus* (EC), *Escherichia coli* (*E. coli*), *Klebsiella pneumonia* (KP), *Acinetobacter baumannii* (AB), and *Pseudomonas aeruginosa* (PA). MDRO species were methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant *Staphylococcus epidermidis* (MRSE), vancomycin resistant *Enterococcus* (VRE), extended-spectrum β-lactamases producing (ESBLs) *Escherichia coli* and *Klebsiella pneumoniae*, carbapenem resistant *Escherichia coli* (CR-*E. coli*) and *Klebsiella pneumoniae* (CR-KP), carbapenem resistant *Acinetobacter baumannii* (CR-AB), and carbapenem resistant *Pseudomonas aeruginosa* (CR-PA).

2.4. Definition. HAIs were defined using the Chinese criteria [15].

2.5. Data Analysis. The patients with HAIs were assumed as the case group and patients without any HAIs as the control group. The total LOS was evaluated for the case. The descriptive statistics and frequency distribution such as mean ($\bar{x}$), standard deviation (SD), and percentage were used.

The differences were analyzed by matched T tests. Due to the skewed distribution of the LOS of the patients, the matched T test was made after logarithmic conversion. All of the statistical analyses were two sided, and $P < 0.05$ was considered significant. Also, SPSS, version 18 was used for data analysis.

3. Results

A total of 49540 HAIs occurred in the 68 surveyed hospitals in 2015, and the number of HAIs at five surveyed sites was 38858. 2119 HAI case-patients and 2119 matched control-patients were identified in this study. The median age of the case group was 59 years, the quartile interval was 29 years, the youngest was 0 years, and the oldest was 98 years old. The median age of the control group was 59 years, the quartile

Table 1: The LOS due to HAI in different hospital levels (days).

Level	n	Case group		Control group		Increased LOS	t	*P*
		$\bar{x}$	SD	$\bar{x}$	SD			
Provincial or ministerial	627	23.2	1.8	12.3	1.9	10.9	24.89	<0.01
Prefectural or municipal	932	21.3	1.9	10.9	1.9	10.4	30.95	<0.01
District or county	560	19.3	2.1	9.6	1.9	9.7	21.94	<0.01
Total	2119	21.3	1.9	10.9	1.9	10.4	45.13	<0.01

Table 2: The LOS due to HAI in different regions.

Regions	n	Case group		Control group		Increased LOS	t	*P*
		$\bar{x}$	SD	$\bar{x}$	SD			
South	226	22.9	2.3	10.3	2.1	12.6	16.24	<0.01
Northeast	117	22.2	2.1	10.1	1.9	12.1	10.22	<0.01
North	501	23.3	1.8	11.9	1.8	11.4	23.25	<0.01
East	720	20.1	1.9	10.2	1.9	9.9	26.61	<0.01
Centre	209	20.6	2.0	11.0	1.9	9.6	14.84	<0.01
Southwest	148	20.5	2.0	11.8	1.9	8.7	9.28	<0.01
Northwest	198	19.9	1.8	11.7	1.8	8.2	12.68	<0.01
Total	2119	21.3	1.9	10.9	1.9	10.4	45.13	<0.01

spacing was 28 years, the youngest was 0 years, and the maximum was 96 years. The ratios of male to female were the same in two groups. 55.11% (1170 cases) were male and 44.89% (953 cases) were female.

The average total LOS of the case group was 21.3±1.9 days and that of the control group was 10.9±1.9 days. The difference between the case and control group was statistically significant ($t = 45.13$, $P < 0.01$). The HAI caused an increase in average stay of 10.4 days.

The LOS due to HAI increased, 9.7-10.9 days, in different levels of hospitals: 9.7 days in district or county hospitals, 10.4 days in prefectural or municipal hospitals, and 10.9 days in provincial or ministerial hospitals, respectively. The LOS in the case groups was significantly higher than that of the control groups ($P < 0.01$) for each level hospital. There was no significant difference in the increased LOS between different hospital levels ($P > 0.05$) (Table 1).

The increased LOS due to HAI in different regions was 8.2-12.6 days. The increased LOS in Northwest China is the shortest while that of South China is the longest. The LOS in the case group was significantly longer than that of the control group in each region ($P < 0.01$). Comparing between regions, we discovered that the increased LOS due to HAI in South China is obviously longer than other regions except the Northeast. The increased LOS due to HAI in Northwest China is obviously shorter than other regions except Central and Southwest China where the difference was statistically significant [($P < 0.05$) (Table 2)].

The increased LOS due to HAI was different in different infection sites which was 6.7-12.8 days. The GI caused the shortest increase in stay of 6.7 days while the BSI caused the longest increase in stay of 12.8 days. The LOS of the patients with different infection sites was significantly longer than that of those corresponding controls ($P < 0.01$). The increased LOS of GI was significantly shorter than that of other sites ($P < 0.05$) but there was no significant difference among LRTI, UTI, SSI, and BSI [($P > 0.05$) (Table 3)].

Among 2119 case-patients, 365 cases detected the studied non-multidrug-resistant pathogens. The average increased LOS due to these bacterial infections was 12.2 days, among which the LOS of KP infection was the most prolonged with 15.5 days followed by EC and SA infection. The LOS of the patients with different pathogen infections was longer than that of the control groups ($P < 0.01$). Compared with SA and KP infection, *E. coli* infection caused significantly shorter LOS ($P < 0.05$); there was no significant difference among other pathogen infections [($P > 0.05$) (Table 4)].

Among 2119 case-patients, the studied MDROs were detected in 381 cases. The average increased LOS due to these MDRO infections was 14 days, among which the LOS of CR-PA infection was the most prolonged which was 26.5 days. The increased shortest LOS was 9.7 days due to MRSE infection. The LOS of the patients with different MDRO infections was longer than that of the control groups ($P < 0.01$). Comparing between different MDRO infections, we found that the increased LOS due to HAI caused by CR-PA is obviously longer than other MDRO infections except VRE and CR-*E. coli* infections ($P < 0.05$); there was no significant difference among other MDRO infections [($P > 0.05$) (Table 5)].

4. Discussion

Healthcare-associated infections (HAIs) affect millions of patients worldwide. HAIs are associated with increased hospital length of stay (LOS), thus increasing the healthcare cost [16], which not only burdens medical resources but also increases patients' suffering and even causes medical disputes.

Table 3: The LOS due to HAI in different sites.

Infection sites	n	Case group		Control group		Increased LOS	t	P
		$\bar{x}$	SD	$\bar{x}$	SD			
BSI	321	25.4	1.9	12.5	1.9	12.8	19.24	<0.01
SSI	405	23.4	1.9	11.5	1.8	11.8	22.77	<0.01
LRTI	537	22.3	1.9	11.1	1.9	11.2	21.71	<0.01
UTI	480	21.2	2.0	10.9	1.8	10.3	20.23	<0.01
GI	376	15.5	1.9	8.8	1.8	6.7	17.84	<0.01
Total	2119	21.3	1.9	10.9	1.9	10.4	45.13	<0.01

Table 4: The LOS due to HAI caused by different susceptible pathogens.

Pathogens	n	Case group		Control group		Increased LOS	t	P
		$\bar{x}$	SD	$\bar{x}$	SD			
KP	47	25.8	2.0	10.2	2.0	15.5	7.34	<0.01
EC	53	25.2	2.0	11.3	1.8	14.0	8.80	<0.01
SA	56	24.4	1.9	10.5	1.8	13.9	9.57	<0.01
PA	34	24.4	1.6	11.8	1.9	12.5	6.66	<0.01
AB	16	27.8	1.9	15.6	1.8	12.2	4.38	<0.01
SE	29	21.5	1.9	10.1	2.0	11.4	5.54	<0.01
E. coli	130	21.0	1.8	11.2	1.7	9.8	11.27	<0.01
Total	365	23.3	1.9	11.1	1.8	12.2	20.73	<0.01

Table 5: The LOS due to HAI caused by different MDROs.

MDRO	n	Case group		Control group		Increased LOS	t	P
		$\bar{x}$	SD	$\bar{x}$	SD			
CR-PA	31	38.8	2.4	12.2	2.0	26.5	6.50	<0.01
VRE	11	35.1	1.9	15.3	1.6	19.8	5.06	<0.01
CR-E. coli	28	27.5	1.9	10.8	1.8	16.8	7.43	<0.01
CR-AB	49	29.4	1.9	13.6	1.9	15.7	6.79	<0.01
CR-KP	26	26.8	1.7	12.6	2.1	14.3	5.75	<0.01
MRSA	60	27.6	1.8	14.3	1.8	13.3	7.29	<0.01
ESBL E. coli	124	22.8	1.9	11.1	1.9	11.7	12.62	<0.01
ESBL KP	38	24.5	1.9	13.2	1.8	11.3	5.40	<0.01
MRSE	18	22.9	2.1	13.2	1.9	9.7	3.19	0.01
Total	381	26.4	2.0	12.4	1.9	14.0	20.36	<0.01

This study found that the increased LOS of HAI was about twice as long as those of the noninfected patients, with an average prolongation of 10.4 days which was close to the results of Sun Jihua [17] and Zhou Chunlian [18].

The increased LOS due to HAI was not related to hospital level but there were regional differences. The clear understanding of the underlining reasons is still lacking.

In this study, the effects of different infection sites on LOS were analyzed. Among them, BSI prolongs hospital stay for 12.8 days. In a multicenter study, involving 69 tertiary-care ICUs of 37 cities in 11 countries, the extra LOS due to CVC-BSI was 9.8 days [19]. In a Brazil study, the increased LOS attributable to BSI was 23.63 days [20]. Through these studies, we can see that BSI can cause longer days of hospitalization. In addition, the extra LOS due to GI was the shortest compared with other infection sites which may be related to the easy treatment of GI and less influence on patients with underlying diseases.

The average extra LOS was 12.2 days for non-drug-resistant pathogens and 14 days for MDRO infection. The average extra LOS of MDRO infection was 2 days longer than that of nonresistant bacteria. Among the non-drug-resistant bacteria, *E. coli* had the shortest extra LOS (9.8 days), but drug-resistant *E. coli* prolonged the length of stay more than 11.7 days. The increased LOS in hospital was also much longer in CR-PA than that in non-drug-resistant PA. There was no significant difference in extra LOS between resistant bacteria and non-resistant bacterial infections in SA, SE, and KP.

In the process of medical treatment, medical staff have paid more and more attention to the impact of MDRO [21–23]. Whether MDRO infection can increase the length of stay in the hospital varies from study to study. Barrasa-Villar JI et

al. [24] thought that hospital infections caused by MDROs did not appear to influence LOS compared with those produced by susceptible strains. However, the extra LOS due to a single MDRO in a specific type of infection was identified in other research studies [7–11].

In our study, the HAIs caused by some MDROs did not lead to longer LOS than those caused by susceptible strains. In some other studies, little or no effects of MDRO on the extended LOS were estimated both in ICU patients [25] and throughout the whole hospital [26]. There are many factors that can lead to longer LOS, such as patients with more comorbidities, patients in serious condition, or ICU stay [27]. Some vulnerable patients with infections need increased care whether they are caused by drug-resistant or susceptible microorganisms that results in prolonged LOS [28].

For hospitals, HAI will lead to the prolongation of average hospital stay which will reduce the number of patients admitted and reduce the hospital's medical income. In addition, in the first 13-18 days after admission, which are the efficient hospitalization days, the hospitalization cost is high, but after 18 days the hospitalization cost is lower than the average hospitalization cost [29]; therefore the treatment of new patients can bring more benefits to the hospital. Studies have found that HAIs occur during hospitalization with an average length of stay being 11 days [18] and that if these infections are controlled, then more patients can be treated.

Increased LOS can lead to more healthcare costs but calculating these costs is complicated due to time-dependent bias [30, 31]. Meredith L. Kilgore et al. [32] thought that it is possible that HAIs may lead to prolonged hospital stay which in turn increases the risk of infection (which was called mixing or endogeneity). In recent years, a number of studies have started to use tendentiousness scores [33] and tool variables [34] to correct this endogeneity but were not very successful.

The majority of articles used time-fixed methods (75%) [16]. Studies using time-fixed methods overestimate additional LOS attributable to HAI. Population heterogeneity, different case definitions, and different microorganisms lead to incomparability of different studies. People have been exploring different research methods, but they still mainly focus on time-fixed research.

One study [35] estimated the excess LOS attributable to HAIs, in which total LOS of patients with and without HAIs is overestimated because of failure to account for the timing of infection. In this study, the differences between the time-fixed and time-varying methods are fully discussed. They showed that the LOS due to HAI in studies using time-fixed method was 9.4 or 12.6 days longer on average than those using time-varying method. LOS due to HAIs is quite different according to the used methods. Overestimation of extended hospital stay may lead to incorrect assumptions of the effect of HAI prevention measures.

This study has two main limitations. First, our case-control matching principles do not take into account comorbidity and severity, which may lead to inaccurate assessment of the impact of HAIs on LOS. Second, we used the time-fixed method which could bias the effect of HAIs [36].

5. Conclusions

HAI can significantly increase the LOS. The increase varies according to hospital level, region, site of infection, and infected pathogen, and it also varies if the pathogens were multidrug-resistant. The HAI caused an increase in stay of 10.4 days. There was no significant difference in the increased LOS between different hospital levels. Comparison between regions shows that the increased LOS due to HAI in South China is obviously longer than other regions except the Northeast. The increased LOS of GI was significantly shorter than that of other sites. *E. coli* infection caused significantly shorter LOS. Comparison between different MDRO infections revealed that the increased LOS due to HAI caused by CR-PA is obviously longer than other MDRO infections.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

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